INSULIN LIKE GROWTH FACTOR-1 INHIBITS CHONDROCYTE APOPTOSIS IN VIVO FOLLOWING EXPERIMENTAL INTRA-ARTICULAR INJURY

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INTRODUCTION:
Following intra-articular injury, one reaction of chondrocytes is programmed cell death (PCD). This results in chondrocyte loss which may eventually contribute to post-traumatic osteoarthritis. A major unsolved challenge in treatment of acute osteochondral injury is preservation of chondrocyte viability and prevention of post-traumatic osteoarthritis. Insulin like growth factor-1 (IGF-1) has been shown to act as an endogenous mediator of chondrocyte survival. Recent studies demonstrate IGF-1 activation of NF-κB has chondroprotective effect via inhibition of Fas and NO mediated apoptosis. In our current study, we test the hypothesis that IGF-1 inhibits chondrocyte PCD following acute osteochondral injury in vivo.

METHODS:
A cooled 2mm drill bit was used to create 2-3mm deep osteochondral injuries to the femoral condyles of 12 adult New Zealand White rabbits. To ensure consistent drug delivery into the knee joints, an intra-articular catheter was placed prior to wound closure. Animals in the treatment group received daily intra-articular injections of IGF-1 (200ng/ml, 0.5cc). Animals in the control group received daily intra-articular injections of vehicle alone. Treatment was initiated immediately after wound closure and was continued for four consecutive days. Following the treatment period, animals were euthanized on post-op day 4. Paraffin embedded sagittal sections were stained for apoptosis by TUNEL. DAPI counterstain was used to determine the total cell count. TUNEL images were captured at 5x at 5.2megapixel resolution, 1um/pixel. Each drill hole was treated as an independent specimen. The area of analysis included the full thickness of articular cartilage extending out to 2.0 mm away from both medial and lateral edges of the injury site. The area of analysis was further subdivided into 0.5mm zones radiating outwards from the injury site with each zone encompassing the full thickness of articular cartilage. For each 0.5mm zone, total cell count and TUNEL positive cell count were measured using semi-automated image analysis software. Statistical analysis was performed using two-tailed unpaired t-tests. The results are presented as the apoptotic index (TUNEL positive/total cells) +/- s.e.m.

RESULTS:
Immediate treatment with 200ng/ml IGF-1 markedly reduced overall chondrocyte apoptosis compared to untreated controls, as measured by TUNEL analysis. Reduced apoptosis was statistically significant in areas adjacent to site of injury at 0-0.5mm (group A) [treated = 0.20 ± 0.02, controls = 0.31 ± 0.04; p<0.05], 0.5-1mm (group B) [treated = 0.09 ± 0.02; controls = 0.20 ± 0.04; p<0.05], 1-1.5mm (group C) [treated = 0.07 ± 0.02; controls = 0.16 ± 0.04; p<0.05], and 1.5-2.0mm (group D) [treated = 0.08 ± 0.02; controls = 0.17 ± 0.03; p<0.01]. At the area of articular cartilage directly adjacent to the injury site (group A), intra-articular treatment with IGF-1 resulted in a 35.5% decrease in chondrocyte apoptosis when compared to untreated control. For the remaining zones of injury chondrocyte apoptosis was 55% decreased (group B), 57% decreased (group C), and 53% decreased (group D). Taken together, these data represent a 50% reduction in chondrocyte apoptosis by IGF-1 in the 2mm zone of articular cartilage adjacent to osteochondral drill injury.

DISCUSSION:
Young chondrocytes must remain resilient to survive in articular surfaces. Not only is cartilage continually subjected to mechanical forces, but no other cell types occupy the matrix that might help maintain chondrocyte viability. Because articular cartilage is poorly vascularized, chondrocytes depend on endogenous mediators such as IGF-1 to diffuse through the matrix and provide survival signals. Based on this role as an anabolic growth and differentiation factor in normal cartilage, IGF-1 represents a potential therapeuetic agent for use in the reduction of chondrocyte apoptosis. These data demonstrate reduction of chondrocyte apoptosis by IGF-1 delivered directly into the knee joint. Additional experiments will need to be performed to determine if increased effects can be obtained with higher concentration of IGF-1 and if treatment leads to long-term cartilage preservation.

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REFERENCES: