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EXPRESSION OF FUNCTIONAL NMDA RECEPTORS IN HUMAN OA CHONDROCYTES

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Purpose: Classical neuronal signalling molecules such as substance P and glutamate have been identified in cartilage and are being shown to have roles in regulation of chondrocyte function. This study looks at the expression and function of the metabotropic glutamate NMDA receptor (NMDAR) in OA chondrocytes.

Methods: Chondrocytes from articular cartilage of human knee joint arthroplasty specimens. NMDAR subunit expression at gene and protein levels was identified by RTPCR and western blotting. NMDAR function was assayed using radolabelled Ca45 uptake and changes in cell membrane potential in response to NMDA and mechanical stimulation (MS; electrophys. only) in the presence and absence of the NMDA antagonists MK801 (noncompetitive), Ifenprodil (NR2B selective) and APV (competitive). Results: NMDAR1, 2A, and 2B, but not NR2C, 2D and 3, were detected at varying levels in OA chondrocytes at both the protein and RNA levels. Assessment of receptor function showed that OA chondrocytes responded to NMDA; this response was decreased by specific antagonists. NMDA induced an increase in Ca⁴⁵ uptake which was reduced to baseline by the antagonists. Both NMDA and mechanical stimulation induced a membrane depolarisation which was decreased or abolished by NMDAR antagonists.

Conclusions: This study shows the presence of functional NMDA receptors composed of NR1, 2A and 2B subunits on human OA chondrocytes. It has been suggested that the NMDA receptor is a mechanosensitive receptor in neurones and the current investigation suggests similar involvement in chondrocyte mechanotransduction.

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RESPONSE OF CHONDROCYTES TO INJURY IN VIVO

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Purpose: Secondary osteoarthritis (OA) commonly occurs following joint injury. We developed a model system to examine the sequence of events that leads to osteoarthritis subsequent to traumatic injury. The goal of the current study was to determine the effect of a single blunt impact on cartilage in vivo in young rabbits. While most studies on cartilage impact have used explants of cartilage, our study is one of the first to examine the effects of traumatic injury in vivo.

Methods: 54 New Zealand white rabbits 3 months of age were subjected to blunt injury equal to 70% and 90% of the cartilage fracture threshold (Borrelli et al., 2002). Cartilage was harvested at 0, 1 and 6 months post-injury. Outcome measures were histology to detect proteoglycan and DNA, immunohistochemistry to determine the presence of proteins, in situ hybridization to mRNA to measure cellular matrix synthesis, and TUNEL to detect DNA fragmentation.

Results: At six months, all animals showed visual signs of OA including degenerated cartilage: no osteophyte was observed. At 1 month after injury, cells in the impact area lost BMP-2 staining, while cells and matrix surrounding the impact zone were positive for BMP-2. To determine whether these cells were actively synthesizing type II collagen, mRNA was assessed by in situ hybridization. At 1 and 6 months, only cells actively synthesizing BMP-2 were positive for type II procollagen mRNA. To determine the fate of these cells, tissue was stained with

hematoxylin and eosin to detect nuclei and TUNEL to detect DNA breakdown. At 1 month. cells in the impact zone were intact with intact nuclei, and, although most cells had intact DNA, some TUNEL staining was observed in the upper 1/3 of the cartilage. At 6 months, cells in the lower half of the impact zone lost their nuclei and about 30% of the cells in the lower half were positive for TUNEL staining. Extensive cartilage degradation and proteoglycan loss was observed in the impacted regions where cells were no longer present. No significant difference was observed between the 60% and 90% impact levels. These results indicate that chondrocytes from young animals are surrounded by BMP-2-containing extracellular matrix and actively synthesize matrix molecules. After a single impact, cells in the impact zone loose their ability to synthesize matrix potentially due to the loss of signaling by BMP-2. Subsequently, cells undergo DNA fragmentation and are lost.

Conclusions: The results from this study indicate that posttraumatic arthritis may be different from primary arthritis. Hallmarks of human primary OA are the presence of osteophyte, specific enzyme-induced matrix degradation, and a high degree of anabolic activity. The occurrence of OA is thought to be due to the response of chondrocytes to the injury with active degradation of the collagen and proteoglycan of the extracellular matrix leading to cartilage degeneration. The results from this study demonstrate that young cartilage with actively synthesizing cells, is susceptible to degeneration following severe injury. Importantly, rather than attempting to repair the damage, the cellular response is passive and occurs in a stepwise manner: first, cells lose their capacity to synthesize matrix, then die, then the matrix degenerates. The lack of the formation of osteophytes is consistent with this passive degeneration. These studies open up an entirely new avenue for potential treatments of cartilage injury following trauma in that renewal of the biosynthetic capacity may be targeted.

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CYTOPROTECTIVE AGENTS (IGF-1 AND JNK-II) EFFECT ON ARTICULAR CARTILAGE UNDERGOING MECHANICAL CHONDROPLASTY

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Purpose: Partial thickness articular cartilage lesions are a common pathology in sports medicine and usually are a consequence of trauma from a sports injury. Mechanical chondroplasty is one technique used to smooth damaged articular cartilage but it may also induce metabolic damage to the surrounding cartilage. Insulin-like growth factor (IgF-1) and c-Jun N-terminal kinase inhibitor (JNK-II) may provide cellular support to the surrounding cartilage and enhance metabolic activity, if administered before or after chondroplasty.

Methods: Eight young bovine knees were obtained and divided into two groups. Group 1; knee condyle quarters were immersed in media containing either IgF-1 at 25, 50 or 100ng/ml or JNK-II at 10, 25, 50uM and incubated at 37°C for 24 hours. After 24 hours, chondroplasty was performed on the condyle quarters. Explants were cut (20-30 mg) and placed in 1mL media without IgF-1/JNK-II and incubated at 37°C until assayed. Proteoglycan synthesis (PG) was measured on Day 2, 4 and 8 using a dimethyl-methylene blue (DMMB) assay. Group 2; chondroplasty was performed immediately and explants were cut and placed in media containing either IgF-1 at 10, 25 or 50ng/ml or JNK-II at 5, 10 or 25uM. PG was measured on Day 1, 3 and 7.