Introduction: Mechanical loading plays an important role in the normal homeostasis of articular cartilage, but loading has also been implicated as a factor in the onset and progression of joint disease [1]. Moderate exercise increases proteoglycan content and cartilage thickness. Abnormal joint loading (e.g., due to pain, immobilization, instability) leads to changes in chondrocyte activity that are associated with cartilage degradation and the progression of osteoarthritis or rheumatoid arthritis [2]. The molecular pathways involved in the transduction of mechanical stress to an intracellular signal are not fully understood. These pathways may be targets of pharmaceutical agents aimed at modifying disease progression.

COX2 inhibitors are rapidly replacing the non-specific non-steroidal anti-inflammatory drugs in the treatment of arthritis and sports injuries due to their potent anti-inflammatory actions and minimal side effects [3]. COX2 activity is the rate limiting step in the synthesis of prostaglandins. There is increased prostanoid release from arthritic compared with non-arthritic cartilage [4]. Cytokine stimulation of cartilage leads not only to increased COX2 activity but also increased NOS (nitric oxide synthase) activity [4]. NO can modify PGE2 production in response to cytokines, and there are interactions between the NO and prostanoid pathways in articular chondrocytes. Inhibition of NO release leads to enhanced release of PGE2 in articular cartilage [5].

The goal of this study was to determine if mechanical stress influences PGE2 production by chondrocytes through an NO-dependent mechanism. We quantified the effects of intermittent mechanical stress on PGE2 production and COX2 expression in explants of porcine articular cartilage and the effect of the NOS2 specific inhibitor, 1400W.

Materials and Methods: Full thickness explants of articular cartilage (5mm diam.) were dissected from the femoral condyles of 2 yr old female pigs and cultured in DMEM with 10% heat inactivated fetal bovine serum, non-essential amino acids, HEPES, penicillin and streptomycin. Test and control explants were paired from adjacent sites in the joint. Prescribed intermittent compressive loads at 0.5 Hz (1sec on, 1 sec off) were applied to 24 explants simultaneously using a modified version of the Biopress system™ (Flexcell International). Explants were placed into individual compression wells in 1 ml of culture medium and allowed to equilibrate under a 10 gf tare load for 1 hr. All experiments were performed at 37°C and 5% CO2, 95% air. Intermittent compression at a magnitude of 0.1 MPa was applied for either 1 hr followed by 23 hrs recovery or for 24 hrs. All controls were cultured in an unloaded state. The effects of two inhibitors were tested, the selective COX2 inhibitor NS398 (100 µM, Alexis) and the selective NOS inhibitor 1400W (2mM, Alexis). PGE2 was measured using the PGE2 ELISA kit (R&D Systems) and NOx (total nitrite and nitrate) using the total NO kit (R&D Systems) and normalized to original tissue wet weight. Explants were disrupted in CHAPS buffer and the cytosol was collected and assayed for protein. Immunoblots were carried out using a monoclonal anti-COX2 and anti-COX1 (Transduction Laboratories) and detected by chemiluminescence (Amersham). Cell viability was measured following the different loading regimens using a fluorescent live/dead assay (Molecular Probes). Statistical analysis was performed by Anova with Duncan’s multiple range test.

Results: PGE2 release into the culture medium was significantly increased in response to both 1 hr intermittent compression, 0.5 Hz followed by 23 hrs recovery and also 24 hrs intermittent compression (p<0.05). PGE2 release after compression was cumulative with the greatest rate of release within the first 4 hrs of the recovery period. Immunoblot analysis showed increased COX2 expression in response to intermittent compression. COX1 protein was not detected. The COX2 inhibitor, NS398, completely inhibited PGE2 production in response to mechanical compression.

NO production was significantly increased (p<0.01) after 24 hrs intermittent compression (Fig 1) but not after 1 hr intermittent compression, followed by 23 hrs recovery. Inhibition of NOS2 induced a significant further increase in PGE2 production in response to compression (Fig 1). Compression in the presence of the COX2 inhibitor for 24 hrs caused inhibition of both PGE2 and NO production. No loss of chondrocyte viability was observed.

Discussion: Physiological magnitudes and frequencies of mechanical stress significantly increased COX2 protein expression and PGE2 production from porcine articular cartilage in vitro. Inhibition of COX2 was associated with decreased NO production, whereas inhibition of NOS activity increased PGE2 production. The mechanical regimens used have been associated with increased proteoglycan synthesis in our previous studies [6]. Our finding of further increased PGE2 production in response to compression in the presence of a specific NOS2 inhibitor support the hypothesis that NO plays a negative role in regulating COX2 synthesis in response to stress. Similar findings were observed on the spontaneous release of PGE2 from human osteoarthritic cartilage in the presence of the non-selective NOS inhibitor L-NMMA [5]. However others find NOS2 inhibitor inhibits PGE2 production [7]. These differences could be attributed due differences in the culture techniques or the effectiveness of the NOS inhibitors used. Inhibition of both PGE2 and NO induced by mechanical compression by COX2 inhibitors has important clinical implications, suggesting that COX2 inhibitors may have a role in prevention of arthritic lesions. Furthermore, our findings indicate that significant interactions exist between the NO and PGE2 pathways, suggesting that the clinical use of either NOS2 or COX2 inhibitors for arthritis may have significant effects on the physiological response of chondrocytes to their mechanical environment.

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