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The influence of mechanical compression on the induction of osteoarthritis-related biomarkers in articular cartilage explants

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Summary

Objective: Macromolecules of the articular cartilage extracellular matrix released into synovial fluid, blood, or urine can serve as potentially useful biomarkers of the severity of osteoarthritis (OA). Biomechanical factors play an important role in OA pathogenesis, yet their influence on biomarker production is not well understood. The goal of this study was to examine the hypothesis that dynamic mechanical stress influences the release of these biomarkers from articular cartilage.

Methods: Explants of porcine cartilage were subjected to dynamic compression at 0.5 Hz for 24 h at stresses ranging from 0.006 to 0.1 MPa. The concentrations of cartilage oligomeric matrix protein (COMP), keratan sulfate (KS measured as the 5D4 epitope), total sulfated glycosaminoglycan (S-GAG), and the KS (keratanase-digestible) and chondroitin sulfate (CS) (chondroitinase-digestible) fractions of S-GAG were measured. Radiolabel incorporation was used to determine the rates of proteoglycan and protein synthesis.

Results: The magnitudes of mechanical stress applied in this study induced nominal tissue strains of 4–23%, consistent with a range of physiological to hyperphysiologic strains measured *in situ*. COMP release increased in proportion to the magnitude of dynamic mechanical stress, while KS, CS and total S-GAG release increased in a bimodal pattern with increasing stress. Protein and proteoglycan synthesis were significantly decreased at the highest level of stress.

Conclusion: Mechanical stress differentially regulates the turnover of distinct pools of cartilage macromolecules. These findings indicate that mechanical factors, independent of exogenous cytokines or other stimulatory factors, can influence the production and release of OA-related biomarkers from articular cartilage.

Key words: Proteoglycan, Cartilage oligomeric matrix protein, COMP, Keratan sulfate, Chondroitin sulfate, Biomechanics, Mechanical compression, Arthritis.

Introduction

Osteoarthritis (OA) is a painful and debilitating disease of the synovial joints characterized by degenerative changes in the structure, composition, and mechanical function of the articular cartilage, subchondral bone, and other joint tissues[1]. A hallmark of the disease is the progressive degradation of the articular cartilage extracellular matrix that occurs, despite the increased biosynthetic activity of the chondrocytes, suggesting that the normal balance of anabolism and catabolism of the extracellular matrix has been disrupted[2–4]. The extracellular matrix consists primarily of type II collagen[5] and the large aggregating proteoglycan, aggrecan[6]. Aggrecan consists of a protein core to which are attached the sulfated glycosaminoglycan (S-GAG) chains, keratan sulfate (KS) and chondroitin sulfate (CS)[7]. Smaller amounts of other collagen species (e.g., types VI, IX, and XI), smaller proteoglycans, and other macromolecules such as cartilage oligomeric matrix protein (COMP), hyaluronan, fibronectin, and tenascin are also present in the extracellular matrix[8].

These extracellular matrix molecules or their fragments can be useful as biological markers, or "biomarkers", to assess the severity of joint disease by minimally invasive means[9–12]. Biomarkers of arthritis are defined as macromolecules originating from joint structures whose levels in synovial fluid, blood, or urine reflect locally occurring metabolic events in the joint[12]. Several of these molecules have been investigated as potential biomarkers of matrix metabolism in OA. Monoclonal antibodies (mAbs) to cartilage macromolecules and various carbohydrate epitopes in the glycosaminoglycans found in cartilage have been valuable in studying cartilage metabolism, as well as alterations in proteoglycan structure and function under conditions of repair or degeneration[13]. For example, COMP is a glycoprotein composed of five identical subunits[14] that has been identified as a potential marker of the severity and progression of OA. The concentration of COMP in the serum or synovial fluid increases in clinical and animal models of OA in relation to the severity of disease as well as the number of joints affected[10,15–21]. The mAb 5D4 recognizes KS, and reacts to an epitope of seven repeating units of disulfated disaccharides[22]. Increases in the synovial fluid and serum levels of 5D4 are associated with OA[10,23–26]. A more general

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measure of proteoglycan, total S-GAG, also increases in synovial fluid in clinical[7,27] and animal models[28] of OA.

Despite the extensive consequences of OA, the etiopathogenesis of this disease is not fully understood and appears to involve a complex interaction of biomechanical stress as a cofactor together with the local biochemical environment[1,29–31]. Both clinical and animal studies have provided strong evidence that mechanical factors can initiate and contribute to the imbalance of cartilage metabolism in OA. Alterations in the normal pattern of joint loading may predispose to OA and may be caused by a variety of factors such as immobilization, joint instability, overuse, trauma, injury, or obesity[32–34]. Studies of OA progression following meniscal or ligamentous instability link alterations in joint loading and kinematics to specific biomechanical changes at the tissue level that may predispose the joint to OA, such as decreased cartilage stiffness (tensile and compressive moduli) and increased hydraulic permeability[35–37].

To better understand the role of mechanical stress on articular cartilage, several *in vitro* models have been developed that allow the application of mechanical stress to articular cartilage in a controlled biochemical environment. In such models, continuous or intermittent compression can alter the biosynthetic rates of proteoglycan and collagen[35,38–47]. The consensus of these studies is that static compression suppresses matrix biosynthesis, while cyclic and intermittent loading can either stimulate or suppress matrix synthesis, depending on the frequency or magnitude of loading. High rates or magnitudes of stress can induce an "injurious" response that has been associated with increased degradation, cell death, and the production of matrix metalloproteinases[40,48–52]. These responses have been reported over a wide range of loading magnitudes and frequencies, and exhibit a stress–dose dependency[53].

Evidence exists for an interaction between mechanical stress and OA-related biomarker release. *In vivo* studies show that physical exercise (running on a treadmill for 60 min or playing soccer for 90 min) can increase the levels of the 5D4 epitope[54], as can running in horses[55]. However, other studies have reported no statistically significant difference in serum KS levels in marathon runners (before and after a 42-km marathon)[56,57] or in patients with arthritis in response to a more chronic (3 months) conditioning activity[58]. A recent study has shown that serum COMP levels are increased immediately following 30 min of walking in healthy subjects[59]. Several *in vitro* studies also provide evidence for a link between dynamic loading of cartilage and the release of various biomarkers of OA. Dynamic compression of cartilage in culture increased immunolabeling for the 3B3(−) epitope of CS, which is believed to be an early indicator of alterations in cartilage metabolism[60] and a marker of cartilage development[41]. Other studies have shown increased 3B3(−) labeling in adult bovine cartilage under a relatively severe compression regimen (5 MPa, 24 h at 0.5 Hz) that was also associated with cell death and tissue damage[40]. Cyclic mechanical compression or tension can upregulate the expression of the COMP gene at different frequencies and magnitudes of stress *in vitro*[61–63]. These few studies suggest an interaction between mechanical stress and OA-related biomarkers; however, the dose–response relationship between the magnitude of dynamic compression and the synthesis and release of these biomarkers is not fully understood.

The goal of this study was to test the hypothesis that cyclic mechanical compression of cartilage alters the release of biomarkers of OA in a manner that depends on the applied stress magnitude. A range of compressive loads was applied to articular cartilage explants to induce physiologic and hyperphysiologic magnitudes of tissue strain (deformation). The release of COMP, the proteoglycan epitope 5D4 (KS), and total S-GAG, as well as the KS (keratanase-digestible) and CS (chondroitinase-digestible) fractions of S-GAG, into the media was measured. Radiolabel incorporation of ^{35}S-sulfate and ^{3}H-proline was used to determine the rates of proteoglycan and protein synthesis, respectively, as a function of stress magnitude.

Materials and methods

ARTICULAR CARTILAGE EXPLANTS

Full-thickness explants of articular cartilage were harvested from the femoral condyles of skeletally mature female pigs (1.5–3 years old) using a 5 mm dermal biopsy punch (Miltex Inc, Bethpage, NY) and separated from the underlying bone. The explants were harvested in adjacent pairs to allow site-matching of control (uncompressed) and compressed specimens to reduce site-to-site variability among explants. Before testing, the explants were allowed to stabilize in culture for 72 h at 37 °C, 5% CO_2, in a 48-well plate containing 1 ml/well of standard culture medium consisting of Dulbecco's Modified Eagle Medium (Gibco, Gaithersburg, MD) with 10% fetal bovine serum (heat inactivated at 56 °C for 30 min) (Sigma Chemicals, St. Louis, MO), 0.1 mM modified eagle medium non-essential amino acids (Gibco), 100 U/ml penicillin/streptomycin (Gibco), 10 mM N-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid buffer (Gibco), and 37.5 μg/ml ascorbate-2-phosphate[64]. Synthetic rates and levels of biomarkers released into the culture media were normalized to the wet weight of cartilage measured before compression.

MECHANICAL COMPRESSION EXPERIMENTS

To determine the effects of mechanical compression on biomarker release, individual explants were dynamically loaded in unconfined compression at stresses of 0.006–0.1 MPa using a modified version of the Biopress™ system, a computer-controlled instrument for compressing tissue explants (Flexcell International, Hillsborough, NC)[64–66]. Briefly, individual cartilage explants were placed in Biopress™ culture plates (Flexcell International) consisting of a Delrin™ chamber attached to the bottom of a flexible silicone rubber membrane. A range of calibrated air pressures was applied to the membrane, and the corresponding compressive stress (σ) applied to the explant was determined from the applied force (F) and the initial cross-sectional area (A) of the explant, where $\sigma = F/A$. The loads were applied at a frequency of 0.5 Hz for 24 h at 37 °C, 5% CO_2. Unloaded (control) explants were incubated for 24 h under the same culture conditions.

MEASUREMENT OF TISSUE STRAIN UNDER DYNAMIC LOADING

To determine the deformation of the explants under the range of applied stresses, a set of explants ($n = 5$) was loaded under the same conditions in an electromechanical materials testing system (EnduraTec ELF 3200, Minnetonka, MN) using a closed-loop, load-controlled test regimen. Each explant was first placed under a tare load of 10 gf and allowed to equilibrate for 1 h. Cyclic compression was applied at 0.006, 0.0125, 0.025, 0.05, or 0.1 MPa at 0.5 Hz