AGING AFFECTS RESPONSE TO CYCLIC TENSILE STRETCH: PARADIGM FOR INTERVERTEBRAL DISC DEGENERATION


*University of Tennessee Health Science Center and +Veterans Affairs Medical Center, Memphis, TN
khasty@utmem.edu

INTRODUCTION

In addition to other factors that play a role in the degeneration of intervertebral discs (IVD), mechanical stress from varying forces is an important modulator of the degeneration. To understand the patho-physiological process of degeneration, an understanding of the mechanical forces an IVD is exposed to in vivo and the responsive changes in gene metabolism resulting from the stress is also needed.

The IVD is a unique structure that consists of a hydrated proteoglycan (PTGL)-rich gel called the nucleus pulposus (NP) surrounded by a tough layer of collagen fibrils called the annulus fibrosus (AF). An important role of a normal IVD is to resist the external loads that cause hydrostatic pressure increases in the NP. This pressure in the form of tensile ‘hoop’ stresses distributing equally in all directions. When dissipating laterally to the AF fibers, these stresses result in strains (deformation per unit length) on annular collagen fibers. Since AF cells are localized in between and attached to consecutive annular lamellae of collagen type I fibers, the strains would induce deformation of the cells and the matrix. Hydrostatic pressure and tensile stress are known to directly play a role in the degeneration of IVD.

Various study methods have been employed to investigate the effects of mechanical stress at a cellular level. A method developed by Barns and Flexcell(Flexcell Intl.) applied stretch stress on a flexible substrate to which cells have attached. In studies involving vascular endothelial and smooth muscle cells, this method (CTS) has been shown to modulate gene expression and matrix protein synthesis and degradation. When utilizing the CTS method, various factors (intensity, frequency, time period, etc.) of mechanical stress need to be considered. It has been shown through previous studies in our lab that a stretch of 8-12% does not significantly influence the environment (temp, medium quantity) of Flexcell plates.

In this study, AF cells from various aged porcine IVD were cultured on flexible-bottomed culture plates while CTS of 10% stretch and 0.5Hz(1 sec on/off) was applied. The effect of CTS was then observed by comparing the group exposed to CTS and the control group after quantifying the changes in the gene expression of AF cells using the real-time polymerase chain reaction (RT-PCR) analysis.

MATERIAL AND METHOD

Source of tissues

IVDs used in this investigation were obtained from female porcine lumbar disc of varying ages.

Cell isolation & culture

Cells from the AF & NP tissues were isolated by 1-2 hours digestion at 37° in 0.05% Pronase(Boehringer Mannheim), followed by overnight digestion at 37° in 0.2% collagenase(Worthington Biologicals) using modified F-12K medium (Invitrogen) with 5% fetal calf serum (FCS, Atlanta Biologicals), 4.8mM CaCl2, and 40mM HEPES buffer (Sigma). The cells were washed in F-12K medium and plated at 1x105 (high density) cells/cm2 onto Bioflex™ plates (Flexcell Intl.) with silicone bottoms coated with gelatin.

Cyclic tensile stress (CTS)

Stretching of the cells was performed at 37° on a Flexcell™ FX4000 operating on a 0.5Hz (1 sec on/off) sinusoidal vacuum signal. A stretch duration of 24hr was programmed for a strain of 10% – 0.5Hz on/1 sec off) sinusoidal vacuum signal.

Analysis

When the cells attach completely and become confluent (at approximately 3-4 days), the medium is switched into a serum free medium. This wash was repeated at least twice during a 6 hours incubation to minimize the effect of the serum. After 24 hours, mRNA was isolated from the cells using a TRizol™ reagent (Invitrogen), assayed for absorbance at 260nm (Pharmacia-Amersham), then reverse transcribed using reagents from Applied Biosystems (ABI) in a programmable thermal cycler (MJ Research). 50 ng of cDNA were amplified in each quantitative PCR reaction. Real-time PCR (7900, ABI) was performed using custom-designed primers and probes specific to the pig genome to determine the ratio of expression. The extracellular matrix genes included type I and type II collagen and proteoglycan. Expression of the proteolytic collagenase gene was also measured.

RESULTS

After the 24 hour CTS application, MMP-1 expression in immature AF was increased greatly compared to the control group with no exposure to CTS. No significant change was observed in the mature and the old groups. In contrast, the expression of type I and type collagen were not significantly different after the CTS exposure in both the immature and mature groups, but the expressions were increased significantly in the old group in comparison to the control group. The level of PTGL was also increased significantly in the old group when compared to the control group. (Figure 1).

DISCUSSION

We have observed that 10% elongation, 0.5Hz CTS upregulates many of the genes for the extracellular matrix in the AF of the mature and the old groups, whereas it downregulates the expression in the immature group. The largest degree of increase was seen in the AF of the old pigs which have the lowest levels of all the groups of mRNA for all the genes except MMP-1 where it has the highest level (data not shown). This result could be useful for determining the appropriate intensity of mechanical stimulation in treating patients with disc disorders. The induction of MMP-1 in the NP of the mature and pig could be used to further investigate how mechanical stimulation contributes its degeneration.

REFERENCE


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