Biologic Response of Rat Intervertebral Disc to Dynamic Compression
-Analysis of Mechanoreceptors in Dynamic Organ Culture System-

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INTRODUCTION

Intervertebral disc (IVD) degeneration is a major cause of low back pain and is considered to be caused by dynamic oscillatory loads against that tissue.[1] Many cell types detect movement or forces via transmembrane receptors, which triggers subsequent remodeling of the cytoskeleton, leading to changes in cell metabolism via numerous downstream events, such as post-translational modifications.[2] Several investigators have demonstrated biologic responses of IVD cells to loading; however the precise mechanotransduction pathways remain unclear. In articular chondrocytes, a number of mechanoreceptors have been identified, such as integrin α5β1 and calcium/calcmodulin-dependent kinase II (CaMKII).[3, 5] CaMKII is one of the components of NMDA signaling complex, and that play a central role in the transduction of calcium signals within the cell and involved in the mechanotransduction pathway in chondrocytes.[5] Integrins (particularly, the α5β1 heterodimer) are well known as a predominant molecule of mechanotransduction pathway in many cell types.[3, 4]

The purpose of this study was to investigate the biologic response of rat intervertebral disc to mechanical stimuli in vitro.

MATERIALS AND METHODS

Animals and Tissue Preparation: All animal procedures were performed under approval and guidance of the Animal Care and Use Committee at the authors’ institution. Sixteen skeletally matured 14-week-old male Sprague–Dawley rats (CLEA Japan, Tokyo, Japan) were used in this study. Under general anesthesia, intervertebral discs between the 7th and 8th caudal vertebrae were taken out aseptically with cranial and caudal side endplates from each rat.

Dynamic Organ Culture System: Each vertebra was sharply cut in parallel plane and put on the bottom of sterilized culturing chamber with DMEM/F12/10%FBS/ascorbate. The dynamic organ culture system was consisted of the chambers, linear stepper motors (PFL35T, NPM, Tokyo, Japan) that loaded compressive force (Fig. 1). The system was driven and monitored by Labview (NI, Texas, USA) program. This culture system was worked in an incubator, and IVD tissues were cultured for 6 days under compression stimuli at 1Hz by 1.3MPa as initial state. This loading force was calibrated and medium was changed every two days. The cultured tissues without compression stimuli were used as control.

Histological analysis: Experimental and control discs sections were stained with safranin-O and graded according the histological scale established by Masuda et al.[6]

mRNA Quantification using real-time RT-PCR: Nucleus pulposus (NP) and anulus fibrosus (AF) were collected from experimental and control disc tissues. Total RNA was isolated from NP and AF cells and real time RT-PCR performed using the gene-specific primers for aggregan, collagen type2-1α, MMP-3 and integrin α5 and β1 subunits, CaMK2 γ and δ subunits as mechanoreceptors respectively (all obtained from Takarabio, Tokyo, Japan). GAPDH was used as the internal control.

Statistical Analysis: Mann-Whitney U test was used to assess the significant difference.

RESULTS

Histological analysis (Fig. 2): Experimental disc showed reduction of disc height and size of NP compared with control disc. Serpentine patterned fibers appeared in stimulated AF (Fig. 2b), and vacuolated cells were decreased in stimulated NP (Fig. 2c, d). Histological score of experimental disc was meanly 7.5 and significantly higher than 4.0 of control disc (p<0.05).

mRNA Quantification (Fig. 3): The expressions of aggregan and collagen type 2 in experimental disc were significantly up-regulated than control disc in NP and AF. In NP, MMP-3 of experimental disc strongly expressed compared to control disc with significant difference. Whereas MMP-3 in AF was up-regulated, it didn’t reach significant difference. The mechanoreceptor expressions of CaMK2-γ were significantly up-regulated by dynamic compression in NP and AF. Furthermore the up-regulation of integrin α5 and β1, and CaMK2-γ in AF was noteworthy.

DISCUSSION

The authors, for the first time, demonstrated that short-term dynamic compression induced the moderate IVD degeneration. The results of present study were considered to reflect early IVD degeneration. Further experimental approach could reveal precise mechanotransduction pathways involved with intervertebral disc degeneration and might contribute a prevention or therapeutic strategy to degenerative disc disease.

SIGNIFICANCE

The biologic response of rat IVD to dynamic compression was revealed from the analysis of mechanoreceptors, extracellular matrix and matrix metalloproteinase.

REFERENCES