Effect of Dynamic Compression on ATP Production and Release by Intervertebral Disc Cells

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INTRODUCTION

Intervertebral disc (IVD) degeneration is known to affect millions of individuals every year but mechanisms involved in IVD degeneration are not fully understood [1]. Cellular biosynthesis of extracellular matrix, which is important for maintaining the integrity of tissue and preventing tissue degeneration, is an energy demanding process. Adenosine triphosphate (ATP), the major energy form in the cell, is mainly generated through glycolysis. Previous studies showed that mechanical loading may affect ATP production in chondrocytes by changing consumption of oxygen and glucose [2], while ATP release from chondrocytes under mechanical stimuli mediated extracellular matrix synthesis through a purinergic pathway [3,4]. Therefore, ATP production may play an important role in regulating extracellular matrix biosynthesis of IVD cells which commonly referred to as chondrocyte-like cells. The objective of this study was to investigate the effects of dynamic compression on the ATP production in porcine IVD cells.

MATERIALS AND METHODS

Lumbar spines were obtained from 4–6 month-old pigs within 2 - 8 h of sacrifice (Cabrera Farms, Hialeah, FL). Tissues were harvested from the regions of annulus fibrosus (AF) and nucleus pulposus (NP) and digested in a collagenase-proteinase solution containing 1mg/ml collagenase (Worthington Biochemical Corp., Lakewood, NJ) and 0.6 mg/ml pronase (Sigma Chemical, St. Louis, MO) overnight at 37 °C. IVD cells were isolated from the tissue digestion and encapsulated in 2% agarose disks (8mm in diameter and 2mm in thickness) at a density of 5x10⁶ cells/ml. These disks were cultured in Dulbecco’s Modified Eagle Media (DMEM; Invitrogen Corp., Carlsbad, CA) containing 10% fetal bovine serum (FBS; Invitrogen) and 1% antibiotics for 24 hours and then used in the compression experiments.

Before compression experiment, the disks were washed with DMEM without FBS. For the compression group, each disk was placed in a testing chamber containing 600µL of high-glucose DMEM and then subjected to a sinusoidal compressive strain (15%) at a frequency of 1 Hz for 4 hours. For the control group, the disks were placed in the same chambers without compression. After 4 hour testing, culture medium was collected and the contents of ATP and lactate in the medium were determined using a luciferin-luciferase method (Sigma) and an enzymatic assay (Sigma). Each disk was incubated in 1 ml of 0.15 M NaCl, 5 mM EDTA (pH 8), 1% Triton X100, and 10 mM Tris-Cl (pH 7.4) at 65 °C for 10 min and then the supernatant was collected for determining ATP and DNA contents after centrifuging at 9000x for 10 min. The DNA content was measured using the Quant-it fluorometric assay (Invitrogen). The measurements of ATP and lactate were normalized by the DNA content of agarose disk.

Student t-test analyses were performed to examine the effect of dynamic compression on productions of lactate and ATP and the differences between AF and NP cells.

RESULTS

Dynamic loading significantly increased ATP release from both NP (p=0.026) and AF cells (p=0.026) (Fig. 1). ATP release from NP cells was significantly higher than from AF cells (p<0.001) (Fig. 1). Significant increases in the lactate production (p=0.048) and total ATP production (the sum of the ATP contents in medium and disk) (p=0.002) by dynamic loading were seen on AF cells, but not on NP cells. AF cells produced more lactate than NP cells (p=0.001) (Fig 2). Total ATP production of NP cells was significantly higher than that of AF cells (p<0.001) (Fig. 3).

DISCUSSION

This study demonstrated that dynamic loading significantly promoted ATP release from IVD cells. This finding was consistent with a previous study on chondrocytes [4] and indicates that IVD cells may be influenced by released ATP through a purinergic pathway [3]. The increase in the lactate production of AF cells by dynamic loading suggests that glycolysis of AF cells is promoted by dynamic loading. It also explains why dynamic loading increased the ATP production of AF cells. Differences found in the productions of ATP and lactate between NP and AF cells suggest that the major metabolic pathways for ATP production are different among these IVD cells. Our future study will further investigate the effects of mechanical loading on the metabolic pathways of IVD cells.

![Figure 1 Comparison of ATP release among different experimental groups (n=6 for AF and n=8 for NP).](image1)

![Figure 2 Comparison of lactate content in culture medium among different experimental groups (n=6 for AF and n=8 for NP).](image2)

![Figure 3 Comparison of total ATP production among different experimental groups (n=6 for AF and n=8 for NP).](image3)

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REFERENCES