INTRODUCTION

Torsion is an important loading mode of the spine. A low degree of axial rotation of the spine at low frequency might increase the spinal length and relieve a subject’s perception of back pain during prolonged sitting, whereas a high torsion angle could cause damage to the disc. Studies have shown that an increased torsional range of motion is associated with clinical symptoms of disc disruption. Moreover, disc prolapse and/or herniation is frequently diagnosed in athletes who participate in sports which involve rotational movement of the spine, e.g. golf players and fast bowlers. Understanding the effects of torsion on the intervertebral disc can improve our understanding of the etiology of disc degeneration and provide insight into prevention and treatment. Therefore, this study was used to analyze the biological response of the intervertebral disc (IVD) to three different degrees of continuous torsion using a bovine organ culture model.

METHODS

Organ culture. Bovine caudal IVDs (diameter 16-22 mm) with endplates were isolated as previously described. A custom-made polycarbonate chamber, which contains two serrated porous titanium plates for securing the disc endplates during torsion, was designed for disc culture during the simultaneous application of compression and torsion. During the entire culture period, a 20N static compression was applied to the disc to prevent swelling of the disc. The discs were kept at 37 °C, 5% CO2 for 5 days. 45 mL Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% Fetal Bovine Serum (Gibco, Switzerland) were supplied and changed every 2 days.

Mechanical stimulation. A combined loading of 20 N static compression and either 0° (control), ± 2° (low), ± 5° (medium) or ± 10° (high) torsion at 0.1 Hz was applied (Mini-Bionix System 858, MTS Systems Corp, Eden Prairie, MN). Loading was applied to the disc for 1 h per day and for four consecutive days, starting from the second day of culture.

Evaluation. Disc tissue was divided into 3 parts: nucleus pulposus (NP) and inner and outer annulus fibrosis (IA and OA) for analysis of cell viability by Live/Dead stain and confocal microscopy, gene expression by real-time PCR and apoptotic activity by caspase 3/7 protein measurement (Promega, Walisellen, Switzerland) (N = 6). Gene expression of the disc tissue, including anabolic genes (Collagen I, Collagen II, Aggrecan, Versican, Elastin), catabolic genes (Adams4, MMP3, MMP13) and small leucine rich proteoglycans (SLRPs) (Biglycans, Decorin, Lumican) were evaluated relative to the static control using the 2-ΔΔct method.

RESULTS

Results showed a significantly higher cell viability in the IA regions of the disc with 2° of torsion compared to static loading controls (LSD post-hoc test, IA: Control vs 2°; p = 0.033). (Table 1) Both anabolic and catabolic genes were up-regulated in all torsion groups relative to the static control group. A 10-fold up-regulation of Collagen II in all torsion groups and Aggrecan in the 10° group was noted. Catabolic genes, including Adams4, MMP3 and MMP13, were all up-regulated 10-100 fold in all torsion groups. A trend of increasing expression of MMP13 with increasing torsion angle was observed in the AF. No statistically significant difference in gene expression between torsion groups was found. Compared with the fresh control, apoptotic activity was significantly increased in the NP of the 5° torsion group.

Table 1: Cell viability of the IVD after 4 consecutive days of torsion for 1h/day at 0.1 Hz. Data represents mean ± SEM. * indicates p < 0.05

<table>
<thead>
<tr>
<th>Torsion</th>
<th>NP</th>
<th>IA</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>71.84 ± 4.30</td>
<td>*66.59 ± 4.66</td>
<td>73.08 ± 3.87</td>
</tr>
<tr>
<td>2° Torsion</td>
<td>81.93 ± 2.28</td>
<td>*78.99 ± 3.55</td>
<td>77.98 ± 4.74</td>
</tr>
<tr>
<td>5° Torsion</td>
<td>73.84 ± 4.99</td>
<td>72.26 ± 4.15</td>
<td>65.20 ± 4.75</td>
</tr>
<tr>
<td>10° Torsion</td>
<td>79.82 ± 2.69</td>
<td>74.22 ± 5.59</td>
<td>68.55± 5.71</td>
</tr>
</tbody>
</table>

DISCUSSION

Our results indicate that repetitive torsion activated matrix remodeling of the IVD in both the NP and AF region. Compared with static loading, torsion up-regulated anabolic and catabolic gene expression. A recent in vivo study using a rat tail model found a significant up-regulation of elastin with torsion, whereas elastin expression was relatively stable in our study. A trend of up-regulation of catabolic genes and a higher apoptotic activity with increasing torsion angle may imply a possible harmful effect with hyper-physiological torsion in the long term. On the other hand, a physiological low angle of torsion (2°) increased cell survival in the inner annulus of the disc, as compared to static loading alone, which may be due to increased nutrition and waste exchange to the inner region of the disc, through matrix expansion on “unwinding”, but this will need further verification through evaluation of the solute transport through the disc under torsion.

REFERENCES


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