The Effect of Lipopolysaccharide on Cell Viability and Cytokine Expression of Bovine Intervertebral Disc Cells

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Introduction: Lipopolysaccharide (LPS) is an endotoxin widely used in pre-clinical models of inflammation. LPS offers an attractive model for inducing inflammation due to its ability to provoke secretion of multiple cytokines (e.g. IL-1β, IL-6, and IL-10) by nucleus pulposus (NP) cells in vitro [1]. This approach may provide an opportunity to study broad aspects of the physiological inflammatory process observed in degenerative disc disease. In addition, LPS can suppress proteoglycan (PG) synthesis and stimulate PG degradation by nucleus pulposus (NP) cells in vitro [2]. While these studies demonstrate the sensitivity of NP cells to LPS, it is unclear whether other disc cells, such as annulus fibrosis (AF) or end plate (EP) cells can respond to inflammatory induction by LPS challenge. The AF is primarily composed of sheets of collagen fibers that resist tension in the disc, whereas the NP and EP are gelatinous and dense cartilaginous tissues respectively, primarily composed of PGs and collagen. The aim of this study is to explore and compare the aspects of the physiological inflammatory process observed in AF and EP cells to LPS challenge. One day after plating, FBS containing media was removed and serum-free DMEM + 10μg/mL LPS was added. Control cell groups were cultured in serum-free DMEM. Cell Viability: On days 1 and 7 post LPS challenge, cells were assayed for viability using Live/Dead viability dye (Molecular Probes). Images of cells were acquired on an inverted fluorescence microscope (Zeiss, 10x), and images were analyzed using ImageJ. Cell viability from LPS group was normalized to control group of each cell type at corresponding time point. Gene Expression: The expression of the cells with or without LPS was examined with quantitative RT-PCR. Total RNA was isolated using RNEasy kit (Qiagen) from plated cells at days 1 and 7 post exposure to LPS. Bovine primers for COL1A, COL2A, Aggrecan, IL-1, IL-6 and GAPDH were designed for assay with the Universal Probe Library from Applied Biosystems. Quantitative reverse transcriptase PCR was performed with ABI Prism 7900 Detector, Eurogentec Master Mix and FAM probe/TARMA quencher reagents (Applied Biosystems), following recommended protocols. Quantitative PCR results were normalized to GAPDH transcript level ΔCt, and expression changes in LPS groups were normalized to control groups using the ΔΔCt method. Statistical analysis was performed using ANOVA & LSD post-hoc test.

Results: Cell Viability: All cell viability is reported normalized to control media conditions. Exposure of disc cells to LPS media resulted in differing effects on cell proliferation and viability. After 1 day of exposure to LPS media, AF and EP cells had cell viability comparable to control group (Figure 1). However, on day 1 post exposure, LPS resulted in ~3.5-fold increase in NP cells compared to control media (Figure 1). By day 7, LPS media had ~ 2-fold increase in cell viability vs. control media (~2X, Figure 1). The level of cell viability was comparable for all cell types at day 7. Gene Expression: Exposure to LPS for 1- or 7- days resulted in changes in gene expression that were comparable for both time points. After 1 and 7 post LPS exposure, Aggrecan expression was diminished in both EP and NP cells, with greater inhibition seen for EP cells (Figure 2). Collagen-2 expression was downregulated in all disc cells at 1- or 7-days post exposure to LPS. Inhibition of Col-2 was greatest in AF cells, followed by EP, then NP cells when exposed to LPS media (Figure 3). Collagen-1 expression was less sensitive to LPS exposure than that seen in Collagen-2 (Figure 4). Changes in pro-inflammatory cytokines were also seen in all cell types due to LPS challenge. While IL-1 was slightly inhibited in AF cells due to LPS exposure, significant upregulation of IL-1 was seen in NP and EP cells (Figure 5). Similarly, IL-6 showed almost no change in AF cells, while NP and EP cells were significantly upregulated due to LPS challenge. However, the level of upregulation by EP and NP cells was comparable for IL-6 (Figure 6).

Discussion: The goal of this study was to compare the effect of LPS on gene expression and cell viability on disc cells isolated from AF, NP and EP. Our results indicate that NP and EP disc cells are most significantly affected by LPS challenge. Exposure of disc cells to LPS resulted in significant cell proliferation as early as 1-day post exposure (Figure 1). Anatomical origin of the cells affected proliferative response, with NP cells having greatest proliferation in LPS vs. control media. The pro-inflammatory cytokines IL-1 and IL-6 were both significantly upregulated in EP and NP cells due to LPS exposure, consistent with previous studies for NP cells [1]. While IL-1 expression was greatest in NP compared to EP, comparable expression was measured for IL-6 in NP and EP cells. Our findings also indicate the ECM genes, aggrecan, collagen-1, and collagen-2 are all downregulated in NP and EP cells due to LPS exposure. This change in expression suggests that LPS can trigger cells to initiate catabolic pathways seen in IVD degeneration [4, 5]. Interestingly, our findings indicate that LPS inhibits aggrecan expression in EP cells more than in NP cells, suggesting that EP tissue may be more susceptible to inflammatory triggered breakdown of the ECM. AF cells were also responsive to LPS challenge; however this response was significantly diminished in comparison to NP or EP cells. Changes in ECM expression by AF cells suggest that LPS challenge did not significantly alter the phenotypic response of AF cells. The results of this study indicate that LPS can be used for induction of pro-inflammatory cascades by disc cells. Upregulation of IL-6, a nociception mediator [6], by disc cells suggests that LPS can be used to study the mechanisms of pain development associated with disc inflammation. Future studies will examine the effect of cell age and anti-inflammatory treatments for potential use in IVD degeneration.


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