

Impact of peroxynitrite on intervertebral disc cells

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INTRODUCTION: Disc degeneration is an age-related process that can start as early as the second decade of life and a variety of inflammatory mediators have been implicated in degeneration and/or pain induction such as cytokines. Special attention has recently been drawn towards reactive oxygen and nitrogen species for their role in disc abnormalities and sensation of dorsal root ganglion neurons. Peroxynitrite is an important tissue-damaging species generated at sites of inflammation and degeneration. It is a highly reactive agent formed *in vivo* from the interaction of superoxide and NO.

The aim of this study was to examine the effects of oxidative/nitrosative stress caused by peroxynitrite on human nucleus pulposus cells.

METHODS: Degenerated human intervertebral disc tissue was analyzed for nitrosylation by immunofluorescence. In addition, human nucleus pulposus cells were isolated from intervertebral discs, expanded and stimulated with a stable peroxynitrite donor (SIN-1). Nitrosylation, NF- κ B nuclear translocation and cell viability were analyzed by immunofluorescence. In addition, gene expression of TNF- α , IL-1 β , IL-6, IL-8 and IL-10 were analyzed by real-time RT-PCR. Statistical analysis was performed with a significance level of $p < 0.05$, using a Student's t-Test.

RESULTS: Degenerated intervertebral disc tissue showed nitrosylation, especially in the nucleus pulposus (Figure 1).

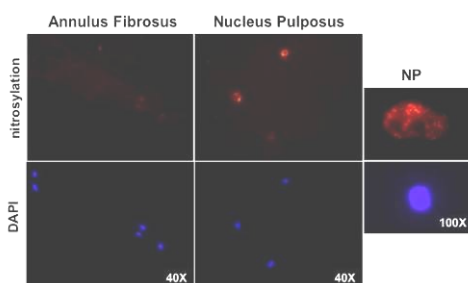


Fig. 1: Nitrosylation in human disc tissue.

Upon stimulation with SIN-1, sustained nuclear translocation of NF- κ B/p65 (Figure 2) and stimulation of IL-1 β , IL-6 and IL-8 expression

(Figure 3) was noted in isolated human nucleus pulposus cells.

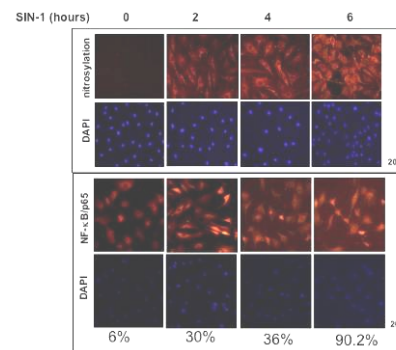


Fig. 2: Nitrosylation and NF κ B translocation in human nucleus pulposus cells upon stimulation with SIN-1 (different time points).

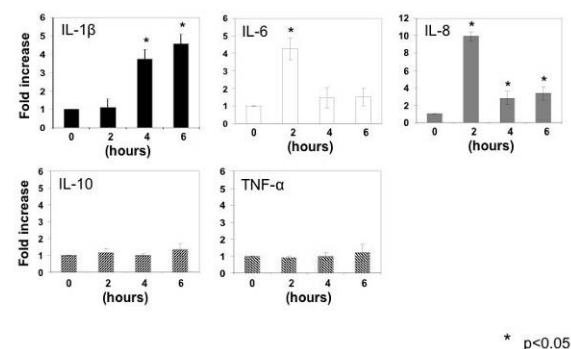


Fig. 3: Changes in gene expression in human nucleus pulposus cells upon stimulation with SIN-1 (different time points).

DISCUSSION & CONCLUSIONS: This study provides evidence that peroxynitrite may play a role in disc degeneration and may induce development of discogenic back pain due to increased synthesis of proinflammatory cytokines. Nuclear translocation of NF- κ B was identified as the potential underlying pathway. Therefore, neutralizing peroxynitrite and its derivatives (e.g. via the use of antioxidants) may be a novel treatment option for discogenic back pain.

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