Title: "Comparative Analysis of Autophagy and Apoptosis-Mediated Intervertebral Disc Degeneration: Effects of Temporary and Sustained Compression in a Rat Model"

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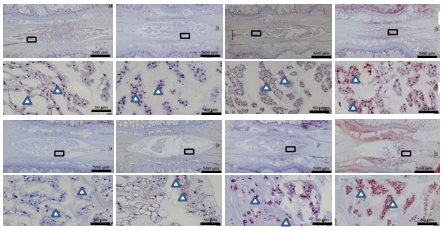
Abstract:

Introduction: Intervertebral discs (IVDs), nutritionally limited organs, rely on autophagy and apoptosis for equilibrium against stressors like compression, trauma, and time. Their interplay maintains homeostasis, but excessive external pressure leads to degeneration. Mechanical compression is a key in intervertebral disc degeneration (IVDD). This study aimed to explore autophagy and apoptosis-induced nucleus pulposus cell (NP) degeneration using temporary and sustained compression to identify optimal modes for studying each process. **Methods:** All animal experiments were conducted by the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals, which followed the ARRIVE guidelines. A total of 32 male Sprague–Dawley rats, aged nine weeks, were utilized in this study. An external fixator was applied to induce both temporary and sustained compression on their tails. Temporary compression was achieved by applying the external fixator for 1 and 2 weeks, whereas sustained compression involved fixator application for 6 and 12 weeks. The study encompassed a range of investigative techniques, including Radiography (X-ray), Magnetic Resonance Imaging (MRI), H&E staining, Masson's trichrome staining (MTS), and immunohistochemistry (IHC) targeting LC3, Beclin-1, P62, Cleaved Caspase-3, MMP-3, and PARP. Furthermore, the study encompassed real-time polymerase chain reaction (RT-PCR) analysis to assess the expression of autophagy-related genes (Beclin-1, LC3, and P62) and apoptosis-related genes (Caspase-3, PARP, and MMP-3). **Results:** Radiography and MRI detected varying disc degeneration, moderate to severe in both experimental groups, proportional to compression duration, notably severe in sustained compression. Contrary to expectations, degeneration patterns differed between temporary and sustained compression. Sustained compression led to central nucleus pulposus (NP) cell degeneration, progressively increasing with compression duration. Temporary compression showed similar but unexpected degeneration. Both groups exhibited elevated autophagic marker expression (LC3, Beclin-1, P62) after 6 weeks, declining notably in sustained compression at 12 weeks. Central NP cells in both groups had heightened Cleaved caspase-3, PARP, and MMP-3 expression, positively correlating with sustained compression duration. Temporary compression showed less apoptotic markers than sustained compression. Autophagy gene expression peaked after six weeks, declining by 12 weeks for both groups. Apoptosis gene expression peaked in sustained compression, positively correlated with longer compression durations, while temporary compression shows lower levels of apoptosis gene expression. **Discussion:** The outcomes of this investigation, sustained compression exerts an influence on nucleus pulposus (NP) cell degeneration, with this effect depending on both the duration and intensity of the applied load. The duration and magnitude of compression load emerge as critical factors that significantly govern the extent of intervertebral disc degeneration. Intriguingly, even temporary compression demonstrated a parallel pattern of NP cell degeneration, counter to the initially hypothesized outcome. Intricate cellular processes, involving autophagy and apoptosis, are engaged in response to both temporary and sustained compression. Autophagy, characterized by the presence of specific marker proteins, was activated in the early stages of compression, subsequently diminishing over time and with the progression of the compression load. In contrast, the activation of apoptosis occurred at a later stage, gradually elevating the expression of apoptotic marker proteins with increasing compression time and load duration. The evidence gathered from this study indicates that autophagy manifests as an initial response following compression, its activity gradually decreases as time and load extend. In contrast, apoptosis appears to be a more delayed reaction, progressively intensifying its impact as the duration and magnitude of compression increase. **Conclusion:** Temporary compression may suit autophagy study; sustained compression may suit apoptosis study in intervertebral disc degeneration. Temporary compression triggers early autophagy-mediated degeneration even with limited compression, while sustained compression triggers slower apoptosis-mediated degeneration needing harsher compression and longer duration. The rat model may not precisely replicate human disc degeneration processes. Nonetheless, findings have a potential impact on addressing spine disorders.



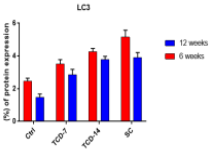


Fig: b: Histogram of LC3

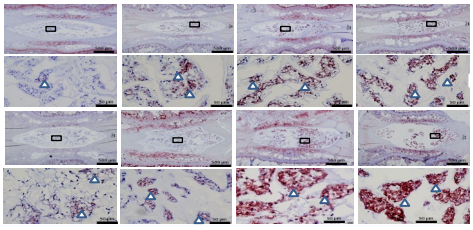


Fig: (A)-MRI results showed moderate to severe degeneration depending on compression duration. (B)-Immunopositive cell of LC3 showed more expression at early stage then gradually decreased after elevating compression duration,(b)-quantitative results of LC3 protein expression. (C)-Immunopositive cell of Cleaved caspae-3 showed late expression which elevated after elevating compression duration.(c)-is quantitative results of Cleaved caspase-3.(n=3)

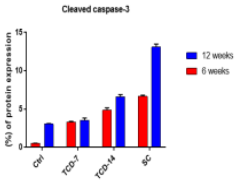


Fig: c: Histogram of Cleaved caspase-3