Abstract:

Which model is advantageous for observing apoptosis or autophagy, saline injection, or external fixator in intervertebral disc degeneration?

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Introduction: The intervertebral disc is a leading avascular organ in the body that may gather nutrition through diffusion. It maintains homeostasis using autophagy and apoptosis to survive unfavorable conditions such as stress and mechanical force. Therefore, excessive force and pressure beyond normal conditions may cause intervertebral disc degeneration. The purpose of this study was to examine which model is advantageous for observing autophagy and apoptosis, external fixator (EF) or saline injection (SI), in rat tail disc degeneration. **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. Sixteen, nine-week-old male Sprague-Dawley rats were treated with 0.9% saline and EF (two-cross Kirschner wires) for six and twelve weeks. In intervertebral disc degeneration, treated discs were dissected to identify the role of autophagy and apoptosis. H&E staining, Masson's trichrome staining, and immunohistochemistry (IHC) for LC3, beclin-1, and P62, as well as MMP-2, MMP-3, and TIMP-1, were used. Furthermore, we conducted real-time polymerase chain reaction (RT-PCR) to observe autophagy-related gene expression (beclin-1, LC3, and P62) and apoptosis-related gene expression (MMP-2, MMP-3, and TIMP-1). **Results:** The EF group showed more insidious NP cell degeneration than the control (Ctrl) group. Degeneration was elevated with increasing compression duration of the EF group, whereas the SI group could not distinguish the margin of annulus fibrosus (AF) and NP cells. LC3, beclin-1, and P62 showed the highest lateral expression, while MMP-2, MMP-3, and TIMP-1 showed up-regulated central expression in both groups. However, the SI group could not recognize the boundary between NP and AF cells. The EF group showed the highest autophagy-related gene expression, whereas the SI group showed lower expression. In addition, the EF group showed more autophagy-accumulating materials than the SI group, which elevated with increasing compression duration. Furthermore, the SI group induced the highest apoptotic gene expression, but the EF group showed the lowest expression. **Discussion:** In this study, we observed distinct degenerative patterns between the EF group and the control (Ctrl) group. The degeneration of NP cells was notably more pronounced in the EF group, and this effect intensified with longer compression durations. In contrast, the SI group could not differentiate between the annulus fibrosus (AF) and NP cells. Examining specific molecular markers, we found that LC3, beclin-1, and P62 displayed heightened lateral expression, while MMP-2, MMP-3, and TIMP-1 showed increased central expression in both groups. Notably, the EF group exhibited the most pronounced elevation in autophagy-related gene expression, with a corresponding accumulation of autophagy-related materials. This accumulation became more prominent with longer compression durations. Conversely, the SI group demonstrated a lower level of autophagy-related gene expression. Moreover, the EF group displayed a lower expression of apoptotic genes compared to the SI group, which exhibited the highest apoptotic gene expression among the groups. These findings collectively indicate that the EF group experiences heightened autophagic responses and a distinct lack of apoptotic gene expression, potentially contributing to the observed NP cell degeneration. In conclusion, our study underscores the differential impact of compression on NP cell degeneration between the EF and SI groups. The EF group exhibited exacerbated degeneration accompanied by heightened autophagy-related gene expression and accumulation of autophagic materials. This contrasts with the SI group, which demonstrated prominent apoptotic gene expression and an inability to differentiate between NP and AF cells. Using rats as a model may not precisely replicate human disc degeneration processes, potentially affecting the relevance of findings. Further comprehensive research is necessary to fully elucidate the underlying factors behind the elevated P62 protein expression in vivo after compression. Furthermore, the limitations of the staining technique restrict our ability to definitively determine if central NP cell death in the SI group occurred via autophagy or apoptosis. These findings illuminate the complex cellular mechanisms underlying intervertebral disc degeneration and provide valuable insights for potential therapeutic interventions.

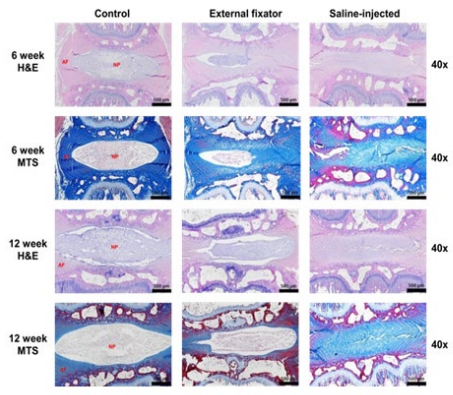

Figure-1: H&E and Masson's trichrome staining results for the EF, SI, and Ctrl groups after six and twelve weeks. Both H&E and MTS showed more insidious NP cell degeneration in the EF group than the Ctrl group after six and twelve weeks, but the SI group showed no margin between AF and NP cells.

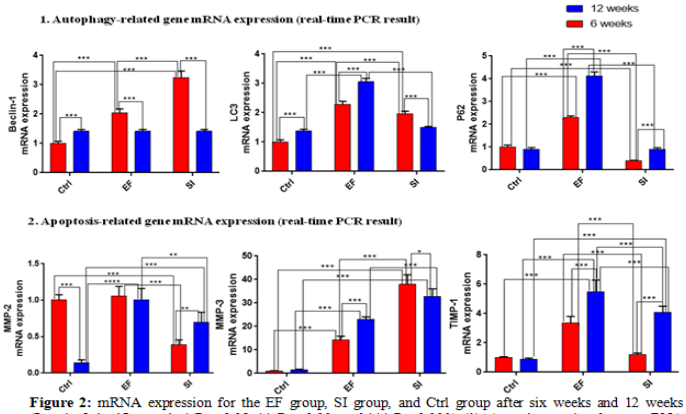

Figure 2: mRNA expression for the EF group, SI group, and Ctrl group after six weeks and 12 weeks (Level of significance is *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$). (1). Autophagy-related gene mRNA expression was highly expressed in the EF group, whereas the SI group showed less expression but higher than the Ctrl. (2) Apoptosis-related gene mRNA expression; the SI group showed maximum mRNA expression, whereas the EF group showed less expression but significantly higher than the Ctrl.