Expression Of Il-1β In Cervical Spondylotic Myelopathy (CSM) Using A New Zealand White Rabbit Model With Spinal Cord Compression

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ABSTRACT

Background: Cervical Spondylotic Myelopathy (CSM) is a severe neurological disorder caused by progressive spinal cord compression, leading to motor dysfunction and paralysis. Inflammatory cytokines, such as Interleukin-1 Beta (IL-1β), are implicated in its pathogenesis.
Objective: This study investigates IL-1β expression in CSM using a New Zealand White rabbit model to better understand its role in inflammation and neurodegeneration.
Methods: A controlled experimental study was conducted on 30 rabbits, divided into three groups: control and two treatment groups subjected to spinal cord compression for 7 and 21 days. Compression was induced using translaminar screws, and IL-1β expression was analyzed through immunohistochemical staining. Statistical analyses included ANOVA, Kruskal-Wallis, and Tukey's post-hoc tests.
Results: IL-1β expression significantly increased with spinal cord compression, with mean values of 3.22 in the control group, 8.89 in treatment group 1, and 11.78 in treatment group 2 ($p < 0.001$). Histopathological analysis revealed marked inflammatory responses, confirming the role of IL-1β in CSM pathophysiology.
Conclusion: IL-1β plays a crucial role in spinal cord inflammation and tissue damage in CSM. Targeting IL-1β with antagonists may provide therapeutic potential in managing CSM-related neuroinflammation.

Keywords: Cervical Spondylotic Myelopathy, IL-1β, inflammation, spinal cord compression, neurodegeneration, cytokines, immunohistochemistry, animal model.

INTRODUCTION

Cervical Spondylotic Myelopathy (CSM) is a severe neurological disorder resulting from progressive compression or irritation of the spinal cord in the cervical region, predominantly affecting the elderly. CSM is a major cause of paralysis, imposing a substantial economic burden on patients, their families, and society. Clinically, CSM presents with symptoms such as neck pain, motor dysfunction in the arms, hands, and fingers. If left untreated, the condition can lead to severe neurological damage, including paralysis and even death.[1]

Despite recent advancements in understanding the pathology of CSM, its prognosis remains poor, and the molecular mechanisms underlying its pathogenesis are still not fully elucidated. In CSM, nerve tissue damage triggers the release of specific cytokines from glial cells, contributing to the disease's progression. Surgical decompression remains the most effective long-term management approach. However, determining the optimal timing for surgical intervention remains challenging. In certain cases, conservative therapy may be considered as an alternative approach.[1,2]

Interleukin-1 Beta (IL-1B) is a key pro-inflammatory cytokine that regulates inflammatory responses following tissue injury. Studies have demonstrated its role in the pathophysiology of CSM, highlighting its potential as a therapeutic target. This study aims to examine IL-1β expression in Cervical Spondylotic Myelopathy (CSM) using a New Zealand White rabbit model with spinal cord compression. The findings may help understand its role in inflammation and neurodegeneration, contributing to potential treatment strategies.[2-4]

METHODS

This study constitutes an analytical experimental laboratory trial employing an animal model to investigate the effects of spinal cord compression on Cervical Spondylotic Myelopathy (CSM). Utilizing a Posttest Only Control Group Design, the research systematically applies controlled spinal compression on New Zealand White rabbits using translaminar screws (4mm diameter, 10mm length), progressively increasing the compression by 1mm per week over a three-week period. The independent variable is the spinal cord compression, whereas the dependent variable is the severity of CSM, quantified through IL-1β expression. Stringent control variables include the homogeneity of test subjects (male rabbits, aged 12 weeks, weighing 2500-3000 grams), uniform environmental conditions, standardized surgical protocols, and consistent histopathological and immunohistochemical staining methodologies.

A total of 30 rabbits are randomly assigned into three groups (10 per group). The procedure involves anesthesia administration using Ketamine hydrochloride and Xylazine, followed by sterile surgical induction of spinal compression at C5. The subjects are subsequently sacrificed through cranio-cervical decapitation, after which spinal cord samples are meticulously harvested for histopathological and immunohistochemical analysis. Tissue processing involves fixation, paraffin embedding, sectioning, deparaffinization, and staining using Hematoxylin-Eosin (HE) and IL-1β polyclonal antibody staining protocols. The expression of IL-1β is assessed under a light microscope at 400x magnification, employing a double-blind evaluation conducted by two independent observers.

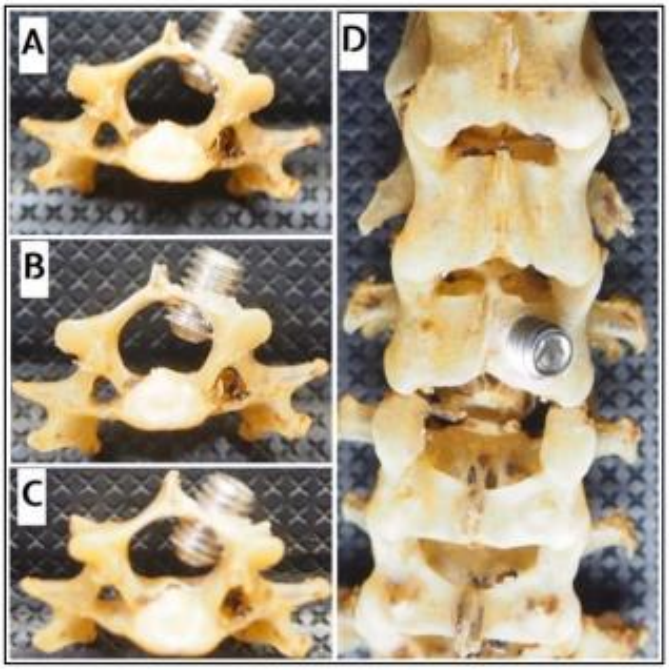

Figure 1. Screw Simulation Model

Statistical analyses include ANOVA for normally distributed data and Kruskall-Wallis for non-parametric data, with Tukey's post-hoc tests for comparative subgroup evaluation. Chi-square or Fisher's Exact tests are applied for categorical data, with statistical significance set at $p<0.05$. Conducted at the Biochemistry-Biomolecular and Pathology Anatomy Laboratories, Faculty of Medicine, Universitas Brawijaya, over a one-month period, this study ensures adherence to rigorous laboratory standards, ethical animal handling protocols, and precise histopathological assessments, contributing valuable insights into the inflammatory mechanisms underlying CSM pathology.

RESULT

This study investigated the effects of chronic progressive spinal cord compression on IL-1β expression using 30 rabbits, divided into three groups: one control and two treatment groups. Immunohistochemical analysis revealed inflammatory processes in the spinal cord of both treatment groups.

Figure 2. Macroscopic Anatomy of the Medulla Spinalis, A: Group A: Without treatment; B: Group 1: Group subjected to compression for 7 days; C: Group 2: Group subjected to compression for 21 days

No mortality was observed in any group, and body weight measurements taken before and after treatment showed no significant differences ($p = 0.898$, 0.830, and 0.283 for the control, positive control, and treatment groups, respectively). IL-1β expression increased with spinal cord compression, with mean values of 3.22 (range: 2–5) in the control group, 8.89 (range: 7–11) in treatment group 1, and 11.78 (range: 10–14) in treatment group 2. The overall mean IL-1β expression across all groups was 7.96 (range: 2–14), with the highest expression observed in treatment group 2 (11.78), indicating a significant inflammatory response. Statistical analysis ($p < 0.001$) confirmed significant differences in IL-1β expression among the groups.

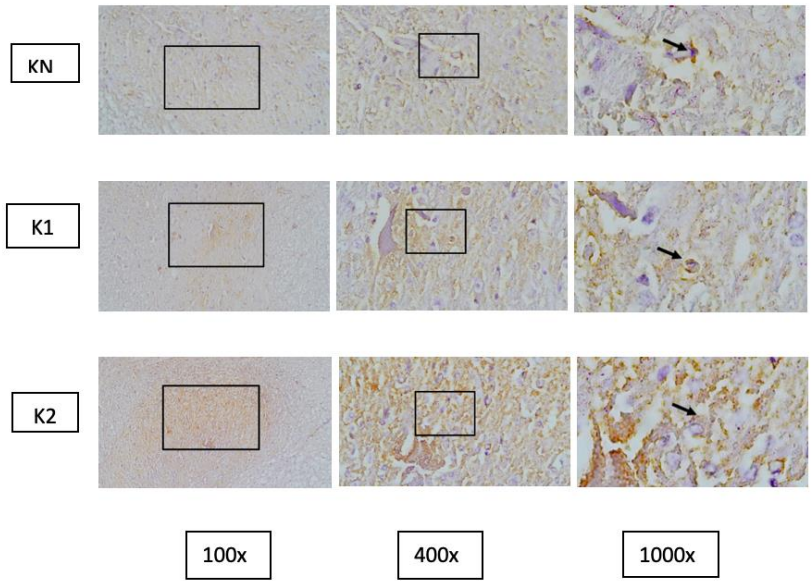

Figure 3. Immunohistochemical staining with IL-1B marker

Post Hoc Tukey's test showed significant differences between the control and treatment group 1, the control and treatment group 2, and between both treatment groups, further supporting the role of progressive compression in increasing IL-1β expression. A homogeneity test also confirmed significant differences among all groups. These findings suggest that chronic spinal cord compression leads to a marked increase in IL-1β expression, supporting its role in inflammation associated with cervical spondylotic myelopathy. The results provide crucial insights into the inflammatory mechanisms involved in spinal cord compression, potentially guiding future therapeutic approaches.

DISCUSSION

This study examines the expression of the cytokine IL-1β in rabbit spinal cord tissue following compression-induced injury using a screw mechanism. Macroscopic observations revealed inflammatory changes in the treated groups, with brownish discoloration at the compression site. Literature on neuroinflammatory responses suggests that trauma triggers a rapid immune reaction, leading to glial cell activation, leukocyte infiltration, and the release of pro-inflammatory cytokines such as IL-1β. These cytokines accumulate at the injury site, sustaining inflammation for months.[5] While inflammation plays a physiological role in clearing necrotic tissue and initiating healing, chronic inflammation can exacerbate tissue damage, contributing to spinal cord pathologies such as chronic pain, neurodegeneration, and traumatic injury. [6,7]

After spinal cord injury, neutrophils infiltrate the lesion and release cytokines, intensifying tissue damage through a self-perpetuating inflammatory cascade. Monocytes and macrophages activate resident microglia, further invading the injured tissue. Pro-inflammatory cytokines, including TNF-α, IL-1β, and interferons, mediate this response, potentially worsening neuronal and glial cell damage. In the spinal cord, TNF-α, produced by macrophages, microglia, and astrocytes, induces microglial activation,

leading to necrosis and apoptosis, thus expanding the myelopathy-affected area. This microglial activation can persist from the acute to chronic phases post-injury, releasing cytotoxic substances such as TNF-α, IL-1, free radicals, and nitric oxide, which contribute to neuronal degeneration in cervical spondylotic myelopathy. [6,7]

The complex interplay of cytokines leads to oxidative stress, cytotoxicity, angiogenesis, scarring, and neurogenesis, with both detrimental and reparative effects. Studies suggest that IL-1 receptor antagonists (IL-1Ra) can block IL-1β receptors, mitigating its pathological effects and improving ischemic spinal cord injuries. Research on rodents shows that anakinra, an IL-1 receptor blocker, offers neuroprotection and prevents cognitive dysfunction in traumatic brain injury models. IL-1β regulates pro-inflammatory responses to tissue injury, with evidence indicating its early involvement in ischemic spinal cord injury progression.[5,8]

CONCLUSION

This study confirms that IL-1β plays a crucial role in spinal cord inflammation, contributing to tissue damage. Targeting IL-1β with antagonists may reduce secondary injury and offer therapeutic potential for spinal cord injuries.

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