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Pirfenidone: An Encouraging Therapeutic Approach For Enhancing Lateral Spinothalamic Tract Function After Spinal Cord Compression Injury

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Abstract

Objective: The objective of this study was to investigate whether intraperitoneally administered pirfenidone could enhance the sensory activity of the lateral spinothalamic tract in rats after compression injury to the spinal cord.
Method: Injury induction at the T7 spinal cord level was achieved using a 70-gram force aneurysm clip in rats. The study involved three groups: Group A received a daily placebo, Group B received a daily dose of 200 mg/kg pirfenidone, and Group C received a daily dose of 500 mg/kg pirfenidone. Subsequently, each group was further divided into two sub-groups, labelled as groups 1 (14 days experimental duration) and 2 (28 days experimental duration), each consisting of 5 rats. On the last day of the experiment, lateral spinothalamic tract activity as pain and temperature sensations in the hind limbs of all rats were assessed.
Results: Scores from the Von Frey test, hot plate test, and acetone drop test exhibited statistical differences both between and within groups. In comparison to non-pirfenidone-treated spinal cord injury groups, those treated with pirfenidone demonstrated a decline in all these scores.
Conclusion: Pirfenidone with its anti-fibrotic and anti-inflammatory action, is likely to enhance the functionality of the lateral spinothalamic tract following spinal cord injury.
Keywords: Pirfenidone, Aneurysm, Spinal Cord Compression.

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1. Introduction

Spinal cord injuries can be broadly categorized as either traumatic or non-traumatic, with traumatic injuries often resulting from accidents, falls, or other physical trauma and can bring about profound and life-changing outcomes.[1] These injuries lead to a cascade of events that cause neuronal damage, inflammation, and scar formation, ultimately disrupting the communication between the brain and the rest of the body. Sensory pathways, including the lateral spinothalamic tract, are particularly vulnerable to damage in spinal cord injuries.[2] These incidents disturb the usual communication channels between the brain and the rest of the body, giving rise to a diverse array of impairments. These include challenges in motor function, deficits in sensory perception, and the onset of persistent pain.[3]

Among the critical sensory pathways impacted by such injuries is the lateral spinothalamic tract, which plays a crucial role in conveying sensations of pain and temperature. Injury to this tract can result in sensory deficits, including numbness, tingling, and altered perception of temperature.[4] Restoring the LSTT function is essential for improving the quality of life for individuals with SCI.[5]

Pirfenidone is an FDA-approved medication primarily used to treat idiopathic pulmonary fibrosis (IPF) due to its anti-fibrotic and anti-inflammatory properties.[6-7] Recent studies have explored its potential neuroprotective effects in various neurological conditions, including SCI.[8] Researchers have hypothesized that Pirfenidone might mitigate the inflammation and fibrotic changes in the injured spinal cord, thereby promoting functional recovery.[9]

2. Materials & Methods

The lab-based experimental research was conducted at the Institute of Basic Medical Sciences, Khyber Medical University in Peshawar, Pakistan from February 2020 to March 2023. Thirty healthy male Sprague Dawley rats, sourced from the National Institute of Health (NIH), were acquired. The sample size was calculated according to the "Resource Equation Approach" These rats, aged 3-4 months and weighing between 250-300 grams, were housed in controlled environments with temperatures ranging from 22-25°C, appropriate humidity levels, and a 12-hour daylight cycle.

The study was conducted after Institutional Advance Study Research Board approval and approval from the KMU Ethics Committee. Institutional Animal Care and Use Committee (IACUC) guidelines were followed in our experiment.

The experimental animals were categorized into groups A, B, and C. Each of these groups was further divided into sub-groups labelled "1" with an experimental duration of 14 days and "2" with an experimental duration of 28 days (n = 5 in each sub-group). Groups A1 and A2 were given dimethyl sulfoxide (DMSO) intraperitoneally daily as a placebo. In groups B1 and B2, a compression spinal cord injury was induced, and daily administration of pirfenidone at a dose of 200 mg/kg/day was performed intraperitoneally using DMSO as a solvent. Groups C1 and C2 underwent compression spinal cord injury and pirfenidone at a dose of 500 mg/kg/day was intraperitoneally administered every day, using DMSO as the solvent.[10]

After administering anaesthesia, a precise incision was performed at the T7 vertebral body level on the rat's back to reveal the spinous process and posterior lamina. The spinous processes of the T7 vertebra, along with the dorsal lamina, were completely removed through laminectomy. Subsequently, a 70-gram force aneurysm clip was carefully applied to the exposed T7 spinal cord segment, maintaining intact meninges. After one minute, the clip was gently removed, and the incision was closed in layers using 15 sutures.[11] Appropriate antibiotics and analgesics were administered to manage post-operative pain and prevent infection.[12] Sensory evaluations were conducted on the 15th day of the experiment for rats in groups A1, B1, and C1, and assessments for rats in groups A2, B2, and C2 took place on the 29th day of the experiment.

The Von Frey manual employed in our experiment serves as the benchmark for determining mechanical pain thresholds in mice and rats. We employed the Ascending Stimulus approach to estimate the mechanical pain withdrawal threshold. This technique is based on the principle of applying Von Frey monofilaments in ascending force until a withdrawal response occurs. The force applied by the Von Frey filament that elicits the withdrawal response is recorded as the mechanical pain withdrawal threshold. The Von Frey monofilaments are calibrated with known force in newtons and weight in grams.[13]

Individually placed on a metal mesh, rats were positioned beneath an inverted glass jar large enough to comfortably accommodate them, ensuring ample space for movement and preventing potential escapes. Throughout testing, the ambient temperature was maintained at room temperature. The rats were allowed 15 minutes of unrestrained exploration to acclimate to the new environment, mitigating the risk of misinterpreting exploratory behaviours as positive responses. Subsequently, Von Frey monofilaments were systematically applied in ascending order, perpendicular to the plantar surface of the hind paw. Each filament exerted a predetermined force, maintained for 2–5 seconds. A positive response was noted when the rat exhibited spontaneous behaviours such as sudden paw withdrawal, licking, or quaking either during filament application or immediately after its removal. The size and force of the applied filament that elicited the response were duly recorded.[14]

The acetone drop test is traditionally employed as a means of assessing cold allodynia, which refers to sensitivity to cold chemical thermal stimuli. This method involves measuring atypical responses triggered by the evaporative cooling impact of acetone. To conduct this test, unconfined rats were placed individually on a metal mesh surface. An overturned glass jar, spacious enough to comfortably accommodate the rat and allow for movement, was positioned over each rat to prevent escape. The ambient temperature during the test was kept at room temperature. The rats were unrestrained and given approximately 20 minutes to acclimate, allowing them to become familiar with their new environment.[15] Subsequently, during periods when the rat was at rest and not engaged in grooming activities, an approximately 50µl volume of acetone was delicately administered to the central region of the hind paw plantar surface. The rat's response to the acetone drop was observed within a 20-second timeframe following its application and was assessed using a 4-point scale. A zero score indicated no response, a score of 1 signified a rapid withdrawal, flick, or stamp of the paw, a score of 2 denoted prolonged withdrawal or repeated flicking, and a score of 3 indicated repeated flicking and licking of the paw. The reactions were timed using a digital stopwatch. Each hind limb's paw plantar surfaces were tested at least three times for individual measurements, and their means were calculated. A break of approximately 5 minutes was introduced between each application of the acetone drop.[16]

The heat threshold in rats was assessed using the hot plate test, which relies on the principle that when rats or

mice are exposed to a heated surface for a duration, they initially exhibit distinctive behaviours such as licking their paws in response to the thermal stimulus, eventually attempting to escape from the confined environment. To conduct the test, we created a rectangular box-shaped electronic hot plate apparatus. The top of the box consisted of a non-adhesive aluminium plate linked to a heat sensor and a digitally controlled thermostat. Below the aluminium plate, an electric heating glass bulb was installed, also connected to the thermostat. The thermostat was digitally set to 50-55°C, and the test was carried out at room temperature.[15] An unrestrained rat was positioned on the preheated metal surface of the aforementioned apparatus. A glass jar, sufficiently spacious to comfortably accommodate the rat and allow ample room for movement, was inverted over the rat to prevent any potential escape. The metal surface temperature was consistently maintained within a range of 50°C to 55°C. A 30-second timeframe served as the cutoff point. The response latency, measured in seconds, was documented as the time taken for the rat to exhibit any nocifensive behaviour within the specified time limit. Nocifensive behaviours, such as forepaw withdrawal, hind paw withdrawal/licking, hind paw stamping, slanting body posture, and jumping, were observed. The duration was meticulously recorded using a digital stopwatch, and the rats were promptly removed upon the initiation of any nocifensive behaviour.[17]

The information obtained from the aforementioned behavioural assessments was inputted and examined using SPSS version 22. Descriptive statistics, including means and standard deviations, were computed. As the data was parametric, so to compare data across groups A1, B1, C1 and A2, B2, and C2, a One-way ANOVA test was employed. Additionally, an Independent Samples T-test was utilized to compare data within the individual groups of A, B, and C.

3. Results

Significant distinctions in Von Frey test scores are evident between subgroups A1 and A2, B1 and B2, and C1 and C2, with corresponding P values of .025, .016, and .008, as illustrated in Figure 1-A. Significant distinctions were observed in the Von Frey test scores among subgroups A1, B1, and C1, with a notable p-value of .006. Likewise, subgroups A2, B2, and C2 exhibited a significant difference in Von Frey test scores, as evidenced by a p-value of .002, as illustrated in Figure 1-B.

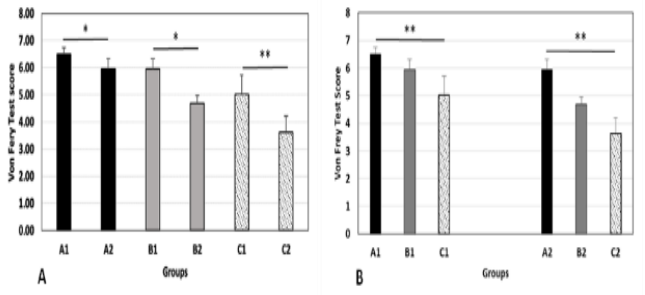

Figure 1: Comparison of mean Von Frey test scores within and between the groups.

The acetone drop test scores exhibited no significant difference between subgroups A1 and A2, with a p-value of .317. Conversely, there was a significant difference in the scores between subgroups B1 and B2 (p = .015) as well as C1 and C2 (p = .042), as depicted in Figure 2-A. Moreover, the comparison of acetone drop test scores among subgroups A1, B1, and C1 yielded a significant result (p = .026). Likewise, a significant difference in scores was observed among subgroups A2, B2, and C2, with a p-value of .01, as illustrated in Figure 2-B.

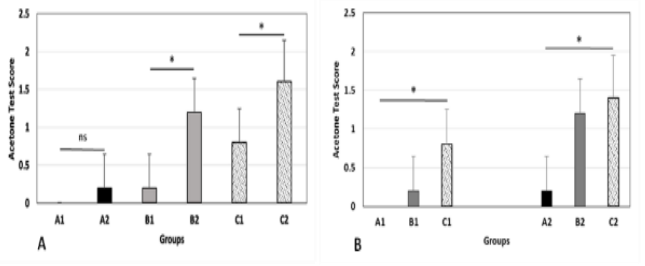

Figure 2: Comparison of mean Acetone drop test scores within and between the groups.

The symbol ⊤ represents standard deviation. Independent sample t-tests and one-way ANOVA revealed p values < 0.05 for all groups except between A1 & A2. Significant p values are denoted by *, while highly significant p values are indicated by **.

There was no significant difference in the hot plate test scores between subgroups A1 and A2, with a p-value of .091. However, a notable significance was observed in the differences between subgroups B1 and B2 (p = .027) and C1 and C2 (p = .015), as depicted in Figure 3-A. The comparison of hot plate test scores among subgroups A1, B1, and C1 also revealed significance, with a p-value of .01. Similarly, a high level of significance was noted in the differences between the hot plate test scores of subgroups A2, B2, and C2, with a p-value of .008, as illustrated in Figure 3-B. It's worth noting that the

decline in hot plate test scores demonstrated an inverse proportionality with the heat threshold recovery.

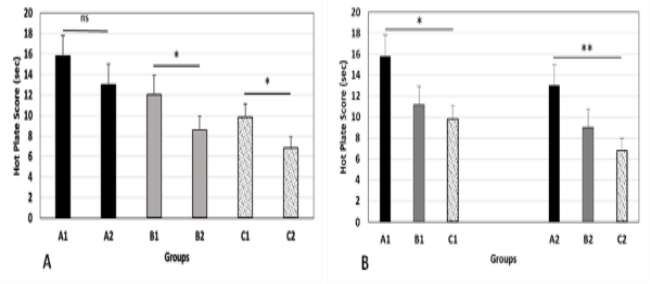

Figure 3: Comparison of mean Hot Plate test scores within and between the groups.

The symbol ┬ represents standard deviation. Independent sample t-tests and one-way ANOVA revealed p values < 0.05 for all groups except between A1 & A2. Significant p values are denoted by *, while highly significant p values are indicated by **.

4. Discussion

Researchers have consistently directed their attention toward addressing spinal cord injuries as a global concern. The primary goal of managing spinal cord injuries revolves around the prevention and mitigation of secondary injuries. A major impediment to the regeneration of damaged axons is the formation of a glial scar, a consequence of secondary injury. As a result, the recovery from spinal cord injuries is often suboptimal, leading to unfavourable outcomes. It is crucial to recognize that the specific motor impairments faced by individuals with spinal cord injuries can vary significantly. Rehabilitation and therapy constitute integral components of the recovery journey for those affected by spinal cord injuries. These interventions strive to optimize functional capabilities, promote independence, and elevate the overall quality of life for individuals grappling with motor disabilities stemming from spinal cord injuries. Furthermore, the incorporation of assistive devices, mobility aids, and adaptive technologies can play a pivotal role in assisting individuals to regain a degree of autonomy and mobility. This research aimed to diminish oxidative stress, inflammation, and fibrosis after spinal cord injury to enhance sensory impairment related to the lateral spinothalamic tract through the application of pirfenidone treatment. Thus, pirfenidone may serve as a viable approach to mitigate neurological deficits and enhance functional recovery post-spinal cord injury, acting as an anti-inflammatory and anti-fibrotic agent. Our findings indicate the effectiveness of pirfenidone in promoting sensory recovery in the lateral spinothalamic tract following spinal cord injury. After reviewing the existing literature, we did not identify any studies that specifically examined and compared the pain sensitivity threshold in rats with spinal cord compression injuries treated with pirfenidone versus those without pirfenidone treatment. Consequently, we compared our study with recent research that explored the effects of various anti-inflammatory, anti-fibrotic, and anti-oxidant interventions on neurological outcomes following spinal cord injury.

The present research analysis corroborates a study detailing the anti-inflammatory and anti-oxidant effects of Tacrolimus (TAC) loaded onto nanospheres composed of polyethene glycol-modified maghemite (PEG-MNs) in a rat model of spinal cord injury induced by weight drop. The findings indicate that TAC-PEG-MNs effectively suppress local inflammatory responses by reducing levels of TNF-a, IL-6, and IL-2, as well as diminishing reactive oxygen species in the spinal cord following injury. Animals treated with TAC-PEG-MNs exhibited a statistically significant and consistent improvement in pain sensitivity thresholds (mechanical allodynia) compared to the control group treated with only TAC or PEG-MNs. This improvement was assessed using von Frey monofilaments on days 5, 10, 15, 20, 30, and 40 post-injury.[18] The investigation conducted by Masoudi and colleagues aligns with our research, affirming the anti-inflammatory and antioxidant properties of astaxanthin. They observed a reduction in the expression levels of COX-2, TNF-α, IL-1β, and IL-6 in a rat spinal cord injury model. Notably, their findings demonstrated substantial improvements in pain threshold following 28 days of astaxanthin treatment post-spinal cord injury. When subjected to the von Frey test, rats treated with astaxanthin exhibited a significant reduction in neuropathic pain compared to the control group rats.[13]

Our current investigation demonstrates a similarity in the recovery of cold sensitivity to the findings of a previous study. In that study, researchers uncovered the anti-inflammatory and anti-oxidant effects of intrathecally administered naringenin in a rat model of spinal cord injury induced by aneurysm clip compression. The results of our study further validate these effects, confirming a notable increase in acetone drop test scores among rats treated with naringenin. Importantly, there is

a statistically significant difference in acetone drop test scores between the group treated with naringenin and the spinal cord injury group treated with a vehicle, particularly on the 28th-day post-injury[17]. The present examination differs from a previous study's analysis, where investigators employed chondroitinase ABC (SABC) as a glial scar degrading agent and utilized photobiomodulation therapy (PBMT) as an anti-inflammatory agent. The study findings indicated a statistically significant enhancement in the cold sensitivity threshold for the chABC, PBMT, and chABC+PBMT groups compared to the SCI+vehicle control group when assessed using a hot plate on the 14th-day post-injury. However, no significant distinction was observed between the three therapeutic groups and the control group on the 28th-day post-injury.[19]

The current findings corroborate the outcomes of a study led by Wang J and colleagues, where they demonstrated the anti-inflammatory and anti-oxidant effects of Tacrolimus (TAC) encapsulated within polyethene glycol-modified maghemite nanospheres (PEG-MNs) in a rat model of spinal cord injury induced by weight drop. The study revealed that TAC-PEG-MNs effectively suppressed local inflammatory responses by reducing TNF-a, IL-6, and IL-2 levels, as well as lowering reactive oxygen species levels in the injured spinal cord. Animals treated with TAC-PEG-MNs exhibited a statistically significant and gradual improvement in heat threshold compared to the control group treated with only TCA or PEG-MNs, as assessed on post-injury days 5, 10, 15, 20, 30, and 40 using a hot plate test.[18] The current analysis of the study aligns with the reported findings, where researchers reveal the anti-inflammatory properties of alendronate. This is evidenced by alendronate's ability to inhibit inflammatory responses induced by spinal cord injury (compression model). The study demonstrates a decrease in heat sensitivity threshold in the SCI+alendronate group compared to the SCI+vehicle group. Additionally, there is a statistically significant difference in hot plate scores between these two groups on the 28th-day post-injury.[20]

5. Conclusion

The mechanisms underlying Pirfenidone's neuroprotective effects are not fully understood, but several hypotheses have been proposed. Pirfenidone may modulate inflammation, reduce scar formation, and promote axonal regeneration, ultimately leading to the functional improvement observed in the lateral spinothalamic tract (LSTT). Additionally, it may have a direct effect on neurons, protecting them from secondary damage and enhancing their survival. The potential benefits of Pirfenidone in enhancing LSTT function following SCI have significant clinical implications. If these results translate to human trials, Pirfenidone could become an essential part of the therapeutic arsenal for individuals with SCI. Improvements in sensory function could enhance patients' overall quality of life and independence by reducing neuropathic pain and sensory deficits. While the road to clinical application is long and complex, the emerging evidence suggesting Pirfenidone's potential to enhance LSTT function following compression spinal cord injuries in rats is an exciting development in the field of SCI research. These findings offer hope for the millions of individuals worldwide who live with the devastating consequences of spinal cord injury. Further studies and clinical trials are needed to validate these promising results and bring us closer to effective therapies for SCI patients.

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Contributions:

N.U.W, A.H, N.K - Conception of study
- Experimentation/Study Conduction
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N.U.W, I.J, - Manuscript Writing
A.H, A.A, N.K, A.I - Critical Review
- Facilitation and Material analysis

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