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Pirfenidone's Potential for Motor Recovery following Spinal Cord Compression Injury

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ABSTRACT

Objective: The aim of this research was to access the ability of pirfenidone in improving motor activity of hindlimbs in rats following compression injury to spinal cord. **Study Design:** Experimental lab-based study. **Settings:** Institute of Basic Medical Sciences, Khyber Medical University Peshawar, Pakistan. **Duration:** From March 2020 to April 2023. **Methods:** Injury induction at the T7 spinal cord level was carried out using a 70gm force aneurysm clip in rats. The rats were divided into three groups: Group A received a daily placebo, Group B received a daily dose of pirfenidone at 200 mg/kg/day, and Group C received a daily dose of pirfenidone at 500 mg/kg/day. Each group was further subdivided into two sub-groups, labeled as Groups 1 and 2, each consisting of 5 rats. The experiment lasted for 14 and 28 days. On the final day, all rats underwent BBB scoring to evaluate the motor activity in their hind limbs. **Results:** BBB scores showed statistically significant differences both between and within groups. Among the SCI groups, those treated with pirfenidone displayed higher BBB scores compared to those without pirfenidone treatment. **Conclusion:** Pirfenidone may improve motor function post-spinal cord injury by reducing fibrosis, preventing collagen buildup in the core of glial scars, and reducing reactive astrocytosis in the scar's outer region. This dual action could promote axonal regeneration and enhance synaptic connections, aiding in neurological recovery.

Keywords: *Pirfenidone, Aneurysm clip model, Spinal cord compression injury, BBB score.*

INTRODUCTION

Spinal cord injuries carry a significant toll on the overall well-being of patients. When these injuries stem from trauma, they become permanent conditions necessitating intervention to mitigate their considerable impact, as they lead to substantial sensory and motor deficits for those affected.[1] There are two pathophysiological stages that may manifest during spinal cord injury: the primary stage and the secondary stage.[2] The spinal cord sustains primary damage when it undergoes initial mechanical compression at the onset of an injury.[3] Among the most prevalent primary injuries are those resulting from compression, such as burst fractures and fracture-dislocation injuries.[4] After the initial primary injury, a secondary injury occurs within minutes, lasting for weeks or possibly months. This secondary injury results in increased levels of neuro-inflammation, oxidative stress, edema, ischemia, the formation of cyst cavities, and the emergence of astrocytic glial scarring.[5] Developmental stages linked with both primary and secondary spinal cord injuries include immediate/instant, acute, sub-acute, intermediate, and chronic phases.[6]

An inflammatory response by phagocytes, macrophage incursion, meningeal and perivascular fibroblast infiltration, and reactive astrocytosis cause the sub-acute damage phase to last approximately two weeks. As oxidative stress increases, neuronal demyelination and apoptosis occur in the intermediate stage. The formation of glial scars in the next stage leads to axonal repair and regeneration suppression, as a consequence of unequal

reconfiguration of hypertrophic astrocytic processes around the lesion and collagen deposition by fibroblasts and reactive astrocytes in glial scar. As a result of limiting the above-mentioned products at the injured site, axonal sprouting and regeneration can be promoted, leading to improved neurological rehabilitation and functional recovery.[7-9]

At start, pirfenidone (PFD) was used as a treatment for helminthic infections and pyrexia. Its tiny molecular size allows PFD to quickly cross the blood-brain barrier and reach most organs when taken orally.[10-11] Pirfenidone exhibits antifibrotic, antioxidant, and anti-inflammatory properties. "Pirfenidone suppresses fibroblast growth, reduces the production of fibrosis-related proteins and cytokines, and promotes the accumulation and retention of extracellular matrix in reaction to growth factors like TGF- and PDGF (platelet-derived growth factor).[12] Several investigations have demonstrated that pirfenidone has anti-inflammatory properties by blocking tumor necrosis factor and interleukin release as well as a variety of other inflammatory cytokines.[13]

METHODS

After receiving approval from the institutional ethical review board (letter reference#:-Dir/KMU-EB/RP/000768), the study was carried out at the Institute of Basic Medical Sciences, Khyber Medical University Peshawar, Pakistan. From the National Institute of Health (NIH), 30 healthy male Sprague Dawley rats were purchased. They averaged 3-4 months of age and weighed 250-300 grams. Rats were kept in controlled environments containing 22-25°C, proper humidity, and 12 hours of daylight. The experiment was carried out in compliance with the guidelines outlined in the National Research Council's Guide for the Care and Use of Laboratory Animals.

Experimental animals were divided into A, B and C groups. Each of these groups were sub-divided into sub-group “1” having experimental duration of 14 days and sub-group “2” having experimental duration of 28 days (n = 5 in each sub-group). Groups A1 and A2 received DMSO (dimethyl sulfoxide) intra-peritoneally as a placebo daily. A compression spinal cord injury was induced in group B1 and group B2, and daily pirfenidone 200 mg/kg/day was administered daily, intraperitoneally using DMSO as a solvent.[14] Group C1 and C2 were subjected to compression spinal cord injury, and pirfenidone 500 mg/kg/day was administered intraperitoneally every day in DMSO as solvent.[14]

Following anesthesia, a precise cut was made on the back of the rat's at T7 vertebral body level to expose the spinous process and posterior lamina. Spinous processes were removed from the T7 vertebra as well as dorsal lamina were completely eliminated by laminectomy. A 70gm force aneurysm clip was applied approximately in the middle of the exposed T7 spinal cord segment with intact meninges. The clip was then removed gently after one minute and the wound was closed in layers.[15] Proper antibiotics and analgesics were given to overcome post-operative pain and infection.

Motor assessments for groups A1, B1, and C1 rats took place on the 15th day of the experiment, while evaluations for group A2, B2, and C2 rats was performed on the 29th day of the experiment. For motor evaluation, we used the BBB (Basso, Beattie, Bresnahan) scoring system, in which the combined efforts of different joint movements of rats were marked on a scale of 0 - 21 points. This scoring system is based on movement and coordination of hind limbs and fore limbs, the ability to walk, trunk position and stability, and the position of the paws and tail.

Using transparent fiber glass sheets, a special box was designed for this scoring. A black non-slippery paint was applied to its floor for clear visibility, and it measured 80 x 80 x 30 cm. To provide proof reading, three digital cameras were installed outside at different angles on 3 sides. Each rat was placed inside the box individually and encouraged to move for four minutes. During moment, rats coordinated joint movements were closely observed and recorded. Based on their moment patterns, all rats were given scores from 0 - 21 by an independent, double-blinded panel of two examiners.[16]

The data was analyzed with SPSS version 22. Descriptive statistics, including the calculation of means and standard deviations, were performed. Group comparisons were made using the Kruskal-Wallis test for between-group differences and the Mann-Whitney U test for within-group differences. Statistical significance was defined as a P-value less than 0.05.

RESULTS

Mean BBB score of sub groups A1 = 0.4 ± 0.5, A2 = 3.8 ± 1.5, B1 = 3.6 ± 1.1, B2 = 10.8 ± 2.4, C1 = 7.2 ± 2.3 and C2 = 14.6 ± 3.0, shown in figures 3.6 and 3.7. BBB scores differed significantly between the groups A1 & A2, B1 & B2, and C1 & C2, by P values of .008, .009, and .0012 respectively. P = .002 represents the high significance of the difference between A1, B1 and C1 in BBB scores. A2, B2 and C2 showed high significance as well, with a difference of .004 between the BBB scores.

According to these above-mentioned values, the motor activity in the hind limbs of pirfenidone-treated group improved more than the non-pirfenidone-treated group. Additionally, it shows that 500 mg/kg/day and a 28-day period of pirfenidone are more effective in improving

motor recovery after spinal cord injury than 200 mg/kg/day for a 14-day period.

DISCUSSION

Researchers have always focused on the treatment of spinal cord injuries as a universal issue. The main objective of treatment in spinal cord injuries is to prevent and reduce secondary injuries. One of the main hindrances to the growth of damaged axons is the glial scar, a product of secondary injury. Consequently, spinal cord injuries do not recover well and have poor outcomes. It's important to note that the specific motor disabilities experienced by individuals with SCIs can vary widely. Rehabilitation and therapy are essential components of the recovery process for people with spinal cord injuries. These interventions aim to maximize functional abilities, improve independence, and enhance the quality of life for individuals with motor disabilities resulting from SCIs. Additionally, assistive devices, mobility aids, and adaptive technologies can play a crucial role in helping individuals regain some level of independence and mobility. The purpose of this study was to reduce oxidative stress, inflammation, and fibrosis after spinal cord injury to improve sensorimotor impairment by pirfenidone treatment after spinal cord injury. So pirfenidone can be used to reduce neurological deficits and improve functional recovery following spinal cord injury as an anti-inflammatory and anti-fibrotic agent. We found that pirfenidone is effective in improving sensorimotor recovery following spinal cord injury.

Our BBB score results confirm the results of Zhang B's recent study, which found significant improvement in BBB scores as well as inclined plate test scores among rats treated with pirfenidone within seven days after a moderate contusion spinal cord injury was induced by weight drop.[17] Same results are reported in another study conducted by Zhang D *et al* on Sprague Dawley rats. They established spinal cord injury by using a modified Allen's method and used puerarin as an anti-inflammatory drug. Assessment of locomotor function confirmed that BBB scores were higher in puerarin 50 mg/kg and 100 mg/kg dose groups than that of the sham group having only SCI and no peurarin treatment. These improvements were noted on 7, 14 and 28 days after induction on spinal cord injury.[18] These results are comparable and similar to our present study results according to the similarity in the anti-inflammatory effects of pirfenidone and puerarin.

Our study is in agreement with recent study conducted by Wang C *et al* who have demonstrated the effectiveness of a bioactive multi-functional citrate-based hydrogel therapeutic system with ultra-long release of mesenchymal stromal cells derived extracellular vesicle (FE@EVs) as an anti-fibrotic and anti-inflammatory therapy for promoting motor functional recovery after induction of spinal cord injury. They have noticed statistically significant high BBB scores in FE@EVs treated rats as compared to only SCI rats after 21, 35 and 49 days of injury induction.[19] Our study is in agreement with the study conducted by Choi Y *et al* in which they demonstrated anti-inflammatory effect of alendronate by suppressing the spinal cord injury induced inflammatory responses, in improving BBB scores of rats having compression spinal cord injury. BBB scores revealed increase in alendronate treated group and a significant difference in between spinal cord injury group and alendronate treated group after 28 days post-injury.[20] Our present study show similarity in motor behavior recovery results with the study conducted by Fakhri S, in which they revealed anti-inflammatory and anti-oxidant effects of intrathecally administered naringenin in improving motor disability following aneurysm clip compression SCI in rats through BBB scoring. BBB score showed a statistically significant rise during the 28 days follow up in naringenin receiving group compared to spinal cord injury group receiving only placebo.[21]

CONCLUSION

Axonal growth and neurological recovery are hindered by glial scarring that develops after spinal cord injury. Motor impairment after spinal cord injury can be reduced by reducing the central fibrotic core and outer reactive astrocytic core of glial scars. It was concluded that pirfenidone, an anti-fibrotic and anti-inflammatory drug, significantly improved functional neurological recovery. This was likely due to its ability to inhibit activation and migration of meningeal fibroblasts, as well as proliferation and reactivation of astrocytes, which prevent glial scar formation following spinal cord injury. In this way, space and a favorable environment are created for the regeneration of axons.

CONFLICT OF INTEREST / DISCLOSURE

The authors assert that they have no conflicting interests.

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The current research work did not receive funding from any agency.

SUBMISSION DECLARATION

The work detailed above has not been previously published, nor is it currently being considered for publication elsewhere. All authors have consented to its publication, and if accepted by this journal, it will not be published elsewhere.

REFERENCES

1. Zhang Y, Al Mamun A, Yuan Y, Lu Q, Xiong J, Yang S, Wu C, Wu Y, Wang J. Acute spinal cord injury: Pathophysiology and

pharmacological intervention. Molecular medicine reports. 2021 Jun 1;23(6):1-8.
2. Anjum A, Yazid MD, Fauzi Daud M, Idris J, Ng AM, Selvi Naicker A, Ismail OH, Athi Kumar RK, Lokanathan Y. Spinal cord injury: pathophysiology, multimolecular interactions, and underlying recovery mechanisms. International journal of molecular sciences. 2020 Oct 13;21(20):7533.
3. Venkatesh K, Ghosh SK, Mullick M, Manivasagam G, Sen D. Spinal cord injury: pathophysiology, treatment strategies, associated challenges, and future implications. Cell and tissue research. 2019 Aug 14;377:125-51.
4. Hachem LD, Fehlings MG. Pathophysiology of spinal cord injury. Neurosurgery Clinics. 2021 Jul 1;32(3):305-13.
5. Alizadeh A, Dyck SM, Karimi-Abdolrezaee S. Traumatic spinal cord injury: an overview of pathophysiology, models and acute injury mechanisms. Frontiers in neurology. 2019 Mar 22;10:441408.
6. Guest J, Datta N, Jimsheleishvili G, Gater Jr DR. Pathophysiology, classification and comorbidities after traumatic spinal cord injury. Journal of personalized medicine. 2022 Jul 11;12(7):1126.
7. Shao A, Tu S, Lu J, Zhang J. Crosstalk between stem cell and spinal cord injury: pathophysiology and treatment strategies. Stem cell research & therapy. 2019 Dec;10:1-3.
8. Karsy M, Hawryluk G. Modern medical management of spinal cord injury. Current Neurology and Neuroscience Reports. 2019 Sep;19:1-7.
9. Quadri SA, Farooqui M, Ikram A, Zafar A, Khan MA, Suriya SS, Claus CF, Fiani B, Rahman M, Ramachandran A, Armstrong II. Recent update on basic mechanisms of spinal cord injury. Neurosurgical Review. 2020 Apr;43:425-41.
10. Ruwanpura SM, Thomas BJ, Bardin PG. Pirfenidone: molecular mechanisms and potential clinical applications in lung disease. American journal of respiratory cell and molecular biology. 2020 Apr;62(4):413-22.
11. Aimo A, Cerbai E, Bartolucci G, Adamo L, Barison A, Surdo GL, Biagini S, Passino C, Emdin M. Pirfenidone is a cardioprotective drug: mechanisms of action and preclinical evidence. Pharmacological research. 2020 May 1;155:104694.
12. Ma HY, Vander Heiden JA, Uttarwar S, Xi Y, N'Diaye EN, LaCanna R, et al. Inhibition of MRTF activation as a clinically achievable anti-fibrotic mechanism for pirfenidone. Eur Respir J. 2023 Apr 27;61(4):2200604.
13. Antar SA, Saleh MA, Al-Karmalawy AA. Investigating the possible mechanisms of pirfenidone to be targeted as a promising anti-inflammatory, anti-fibrotic, anti-oxidant, anti-apoptotic, anti-tumor, and/or anti-SARS-CoV-2. Life Sciences. 2022 Nov 15;309:121048.
14. Schaefer CJ, Ruhrmund DW, Pan L, Seiwert SD, Kossen K. Antifibrotic activities of pirfenidone in animal models. European Respiratory Review. 2011 Jun 1;20(120):85-97.
15. Gokce EC, Kahveci R, Atanur OM, Gürer B, Aksoy N, Gokce A, Sargon MF, Cemil B, Erdogan B, Kahveci O. Neuroprotective effects of Ganoderma lucidum polysaccharides against traumatic spinal cord injury in rats. Injury. 2015 Nov 1;46(11):2146-55.
16. Kim M, Kim KH, Song SU, Yi TG, Yoon SH, Park SR, et al. Transplantation of human bone marrow-derived clonal mesenchymal stem cells reduces fibrotic scar formation in a rat spinal cord injury model. Journal of Tissue Engineering and Regenerative Medicine. 2018 Feb;12(2):e1034-45.
17. Zhang B, Ding Z, Dong J, Lin F, Xue Z, Xu J. Macrophage-mediated degradable gelatin-coated mesoporous silica nanoparticles carrying pirfenidone for the treatment of rat spinal cord injury. Nanomedicine: Nanotechnology, Biology and Medicine. 2021 Oct 1;37:102420.
18. Zhang D, Ma G, Hou M, Zhang T, Chen L, Zhao C. The neuroprotective effect of puerarin in acute spinal cord injury rats. Cellular Physiology and Biochemistry. 2016 Sep 5;39(3):1152-64.
19. Wang C, Wang M, Xia K, Wang J, Cheng F, Shi K, Ying L, et al. A bioactive injectable self-healing anti-inflammatory hydrogel with ultralong extracellular vesicles release synergistically enhances motor functional recovery of spinal cord injury. Bioactive materials. 2021 Aug 1;6(8):2523-34.
20. Choi Y, Shin T. Alendronate Enhances Functional Recovery after Spinal Cord Injury. Experimental neurobiology. 2022 Feb 2;31(1):54.
21. Fakhri S, Sabouri S, Kiani A, Farzaei MH, Rashidi K, Mohammadi-Farani A, et al. Intrathecal administration of naringenin improves motor dysfunction and neuropathic pain following compression spinal cord injury in rats: relevance to its antioxidant and anti-inflammatory activities. The Korean Journal of Pain. 2022 Jul 7;35(3):291.