Pirfenidone Confines Glial Scar and Improves Functional Recovery after Compression Spinal Cord Injury

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Abstract

Objectives: To investigate the anti-fibrotic and anti-inflammatory outcomes of pirfenidone through the intraperitoneal route to improve neurological recovery by reducing glial scar in rats aneurysm clip compression model of spinal cord injury.

Methodology: Thirty male Sprague Dawley rats were randomly assigned into three groups. All rats in all groups went through compression spinal cord injury induction. Group A, was treated with a placebo, group B was treated with pirfenidone 200 mg/kg/day, and group C was treated with pirfenidone 500 mg/kg/day. The experimental duration of 14 and 28 days was used to subgroup each group into groups 1 & 2 respectively (n = 5 in each subgroup). In order to inflict compression spinal cord injury, a 70-gram aneurysm clip was applied for one minute to the T7 level. To evaluate motor activity in the hind limbs of each subject, BBB scoring was used on the last day of the experiment. To determine collagen content in the injury lesions, Masson's trichrome staining was applied to longitudinal spinal cord sections, followed by immunohistochemical staining with an anti-GFAP antibody for evaluating reactive astrocytosis. ImageJ Fiji software was used to quantify collagen deposition and reactive astrocytes.

Results: Between groups and within groups, a statistically significant difference was found in BBB scores, collagen deposition, and reactive astrocytes. There was an obvious upsurge in BBB scores, a reduction in the collagen deposition inside the injury lesion and reactive astrocytes around the injury lesion of pirfenidone-treated groups compared to the non-pirfenidone-treated SCI group.

Conclusion: Pirfenidone reduces collagen in the central core and reactive astrocytosis in the outer core of the glial scar by reducing fibrosis and inflammation. By limiting collagen deposition in injury lesions and inhibiting astrocyte activation, pirfenidone supports functional neurological recovery after spinal

cord injury. Both of these factors play a critical role in inhibiting axonal regeneration succeeding spinal cord injury.
Keywords: Pirfenidone, Aneurysm Clip Model, Reactive astrocytosis, Spinal Cord Compression Injury, Glial Scar.

Tob Regul Sci.™ 2023;9(1): 1253-1263
DOI: Doi.Org/10.18001/TRS.9.1.86

Introduction

Spinal cord injuries are one of the most serious health issues, affecting patients' overall quality of life. Traumatic spinal cord injuries are a lifetime condition that must be addressed to reduce consequences [1]. The primary and secondary phases of spinal cord injury are two connected pathophysiological stages that might occur [2]. When the spinal cord is damaged by the first mechanical pressures from displaced bones, disc materials, and/or ligaments etc, primary damage occurs [3-5]. After a primary injury, there is a secondary injury that lasts for weeks or months, causing more damage to the surrounding spinal cord tissue [3]. Primary and secondary spinal cord injuries progress through certain developmental stages, including immediate/instant, acute, sub-acute, intermediate, and chronic [6].

The sub-acute damage phase lasts about two weeks and is characterized by a strong phagocytic response, macrophages incursion, meningeal and perivascular fibroblast infiltration, and reactive astrocytosis caused by astrocyte activation by inflammatory mediators. In the intermediate phase, oxidative stress-induced apoptosis and neuronal demyelination persist. This is followed by the formation of glial scars, which indicate the suppression of axonal repair and regeneration as a result of the unequal reconfiguration of hypertrophic astrocytic processes around the lesion forming the outer core of the glial scar, as well as collagen deposition by fibroblasts and reactive astrocytes, which forms the central core of the glial scar. This deposition of collagen and chondroitin sulfate proteoglycans (CSPGs), which are released by reactive astrocytes, are the main constituents of axonal regeneration inhibitory extracellular matrix (ECM). Restricting the above-mentioned products at the damage site can promote axonal sprouting/growth and regeneration which can in turn improve functional and neurological recovery after spinal cord injury [7-10].

Pirfenidone (PFD) is a simple pyridine that was first developed as an anti-helminthic anti-pyretic drug. PFD is quickly absorbed in the gastrointestinal tract when taken orally, reaching most organs and crossing the blood-brain barrier due to its tiny molecular size [11]. Pirfenidone's mechanism of action is not entirely understood. However, existing data reveals that pirfenidone has antifibrotic, anti-oxidative and anti-inflammatory characteristics in a range of in vitro and animal models of pulmonary fibrosis. In idiopathic pulmonary fibrosis (IPF), pirfenidone has shown a reduction in inflammatory cell accumulation in response to various stimuli. Pirfenidone inhibits fibroblast proliferation, the generation of proteins and cytokines that are associated with fibrosis, as well as the enhanced formation

and storage of extracellular matrix in response to cytokine growth factors including TGF-β and platelet-derived growth factor [12]. Several investigations have demonstrated that pirfenidone has anti-inflammatory properties by blocking tumor necrosis factor, interleukin release, and a variety of other inflammatory cytokines [13-16].

Materials And Methods

This study was conducted at the Khyber Medical University Peshawar, Pakistan, Institute of Basic Medical Sciences Department of Anatomy, Pathology, and Pharmacology, after approval from the institutional ethical review board and biosafety office. Thirty male healthy adult Sprague Dawley rats, an average of 3-4 months old and weighing 250-300gm, were purchased the from National Institute of Health (NIH). Controlled environmental settings at 22–25 ^{0}C, proper humidity and a "light cycle" of 12 hours were maintained for each rat. All rats were given free access to food. The experiment was carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Grouping

There were three main groups A, B and C. Each group was sub-grouped in 1(14 days experimental duration) and 2 (28 days experimental duration) based on experimental duration (n = 5 in each sub-group). In group A, compression spinal cord injury was performed and DMSO (Dimethyl sulfoxide) was injected intra-peritoneal a as placebo daily. In group B compression spinal cord injury was performed and pirfenidone 200 mg/kg/day dissolved in DMSO was injected intra-peritoneally daily. In group C, compression spinal cord injury was performed and pirfenidone 500 mg/kg/day in DMSO as solvent was injected intra peritoneal daily [17].

Spinal Cord Injury Model

Isoflurane was used as an inhalational anaesthetic agent. After recognizing T7 spinous process, spinous process of the T7 vertebra was removed and laminectomy was performed to completely remove the dorsal lamina. An aneurysm clip having 70gm force was applied approximately in the middle of the exposed segment of the spinal cord with intact meninges and then removed gently after one minute following wound closure in layers [18]. To overcome post-operative pain and infection, buprenorphine as an analgesic 0.05 mg/kg was administered subcutaneously twice every day for the first 2 days and ampicillin 33 mg/kg/day subcutaneous injection was given two times daily for 6 days [19, 20]. Gentle urinary bladder massage of all rats by Crede manoeuvre along with gentle abdominal squeeze was performed twice a day to empty the urinary bladders and to stimulate defecation [21, 22].

End of Experiment

BBB (Basso, Beattie and Bresnahan) Scoring was done by two independent examiners in a double-blinded manner on the last day of the experiment to assess the motor activity of the hind limbs on a scale ranging from 0 – 21 points which signifies progress in recovery and categorizes combine efforts of different joint movements of rats [23]. Intraperitoneal injection of 200 mg/kg pentobarbitone sodium was used for euthanasia according to American Veterinary Medical Association guidelines [24, 25]. The whole vertebral column was taken out along with the spinal cord inside it and was immediately transferred to 10% neutral buffered formalin for 24 hours to fix and make the spinal cord a bit stiff. After 24 hours, 1.5cm spinal cord segments with an injury site in the centre were taken out undamaged from the vertebral column. These segments were then processed and embedded in paraffin wax for sectioning. 5 µm thin longitudinal tissue serial section were made by microtome. Serial sectioning of spinal cord tissues was done from the dorsal to ventral direction.

Staining and Immunohistochemistry

Around 18 slides of each specimen, having three sections in serial order on each slide, were prepared. For collagen fibers quantification in the central core of the glial scar at the injury site, slide number 5, 10 and 15, and to quantify reactive astrocytosis around the glial scar forming the outer core of the scar, slide number 4, 9, and 14 out of total 18 slides were selected from each spinal cord tissue specimen to estimate the average measure of above mention parameters. This selection of slides was based on the selection of serial sections from dorsal, central and ventral parts of the injured spinal cord area, so that the average of reactive astrocytes and collagen deposition can be determined in the whole injury area.

Commercially available Masson's trichrome kit (Catalog #: - 1030, Medilines, Lahore, Pakistan) was used to stain slides for quantification of collagen in the injury site. Standard steps of Masson's trichrome staining procedure were followed [26, 27]. GFAP (Invitrogen, Thermofisher Scientific) monoclonal primary antibody was used in 1:100 dilution for the detection of reactive astrocytes around the damaged area in the spinal cord by standardized immunohistochemistry technique [26, 28, 29].

Microscopy & Statistics

For microscopy and data analysis, Nikon Eclipse 80i microscope was used. Multiple images in specific sequences were taken at 200X magnification, covering a 1200µm diameter area around the centre of the injury which covered the entire injury area and surrounding tissue. These images were then stitched and grouped together using ICE software (Image Composite Editor 2.0). Reactive astrocytes were counted in a 1200µm area from the centre of injury on both sides of the injury and collagen fibers deposited inside injury lesions were quantified by ImageJ Fiji software.

The data were analyzed in SPSS version 22. Means and standard deviations were calculated for descriptive statistics. Kruskal Wallis test was applied to compare the data between the groups and

Mann Whitney U test was applied to compare data within the groups. P value of < 0.05 was considered statistically significant.

Results

The mean BBB score of all groups is shown in figures 1-A and 1-B. Differences in the BBB scores within the groups A1 and A2, B1 and B2, and C1 and C2 showed significance as *P* values for these are *P* = .008, *P* = .009 and *P* = .012 respectively, shown in figure 1-A. The difference between the BBB scores of A1, B1 and C1 showed high significance as *P* = .002. Similarly, the difference between the BBB scores of subgroups A2, B2 and C2 also showed high significance as *P* = .004, demonstrated in figure 1-B.

The mean percentage of injury area occupied by collagen of all groups is shown in figures 1-C and 1-D. Differences in the collagen deposition in injury sites within the groups A1 and A2, B1 and B2, and C1 and C2 showed significance as *P* values for these are *P* = .009, *P* = .016 and *P* = .028 respectively, shown in figure 1-C. The difference between the collagen deposition in the injury site between the groups A1, B1 and C1 and between A2, B2, and C2 showed high significance as the *P* value for both was .002, shown in figure 1-D.

Mean counts of reactive astrocytes of all groups are shown in figures 1-E and 1-F. Differences in the reactive astrocytes count within the groups A1 and A2, B1 and B2, and C1 and C2 showed high significance as the *P* value for all these groups is .009, shown in figure 1-E. The difference between the reactive astrocytes count around the injury site of between the groups A1, B1 and C1 and A2, B2, and C2 showed high significance as the *P* value for both was .002, demonstrated in figure 1-F.

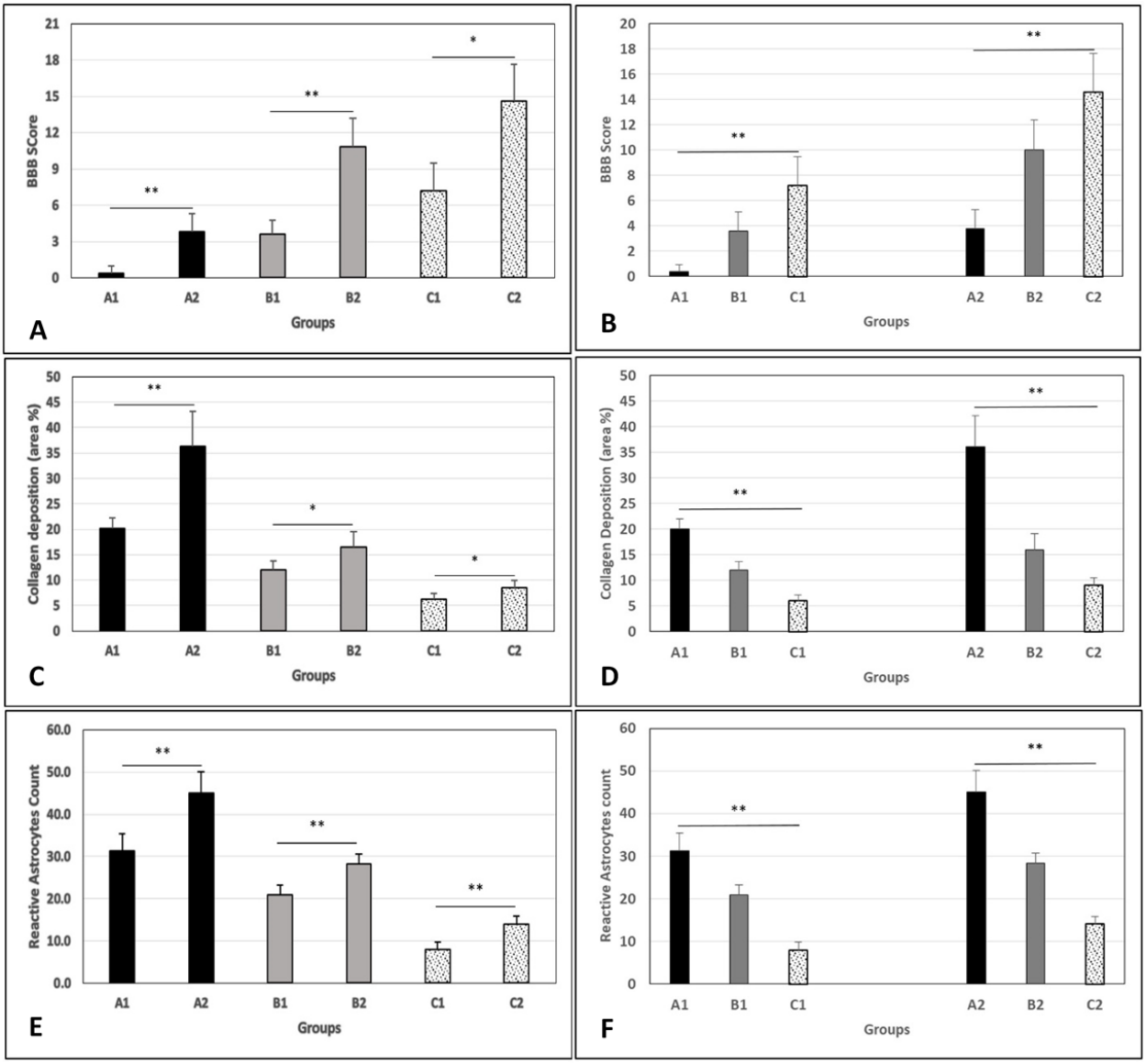

Figure 1:- **(A)** Within the group comparison of mean BBB scores. **(B)** Between the groups' comparison of mean BBB scores. **(C)** Within the groups' comparison of the mean percentage of collagen deposition in the injury site. **(D)** Between the groups' comparison of the mean percentage of collagen deposition in the injury site. **(E)** Within the groups' comparison of mean reactive astrocytes count around the injury site. **(F)** Between the groups' mean reactive astrocytes count comparison around the injury site. **(A1)** Non-Pirfenidone treated group with 14 days experimental duration. **(A2)** Non-Pirfenidone treated group with 28 days experimental duration. **(B1)** 200 mg/kg/day Pirfenidone treated group with 14 days experimental duration. **(B2)** 200 mg/kg/day Pirfenidone treated group with 28 days experimental duration. **(C1)** 500 mg/kg/day Pirfenidone treated group with 14 days experimental duration. **(C2)** 500 mg/kg/day Pirfenidone treated group with 28 days experimental duration. ⊤ denotes SDEV. Mann Whitney U test and Kruskal Wallis test P value <0.05 in all groups.

These above-mentioned values indicate that there was more progress in BBB scores of pirfenidone-treated groups as compared to the non-pirfenidone group. It also reveals the effectiveness of pirfenidone in a higher dose of 500 mg/kg/day and for an extended duration (28 days) in motor recovery after spinal cord injury compared to the lower dose of 200 mg/kg/kg/day for a short duration (14 days). Our results showed that there was more collagen accumulation inside the injured area and increased reactive astrocytosis around the injury site in the non-pirfenidone-treated group as compared to the pirfenidone-treated groups. It also reveals the effectiveness of pirfenidone in a higher dose of 500 mg/kg/day as compared to a lower dose of 200 mg/kg/day in the reduction of collagen secretion and reactive astrocytosis, thus reducing glial scar formation after spinal cord injury. More collagen and reactive astrocytes were noted in 28 days experimental duration groups as compared to 14 days duration group which demonstrates a time-dependent increase in inflammatory changes after spinal cord injury. These changes in collagen and reactive astrocytosis are shown in Figures 2 and 3 respectively.

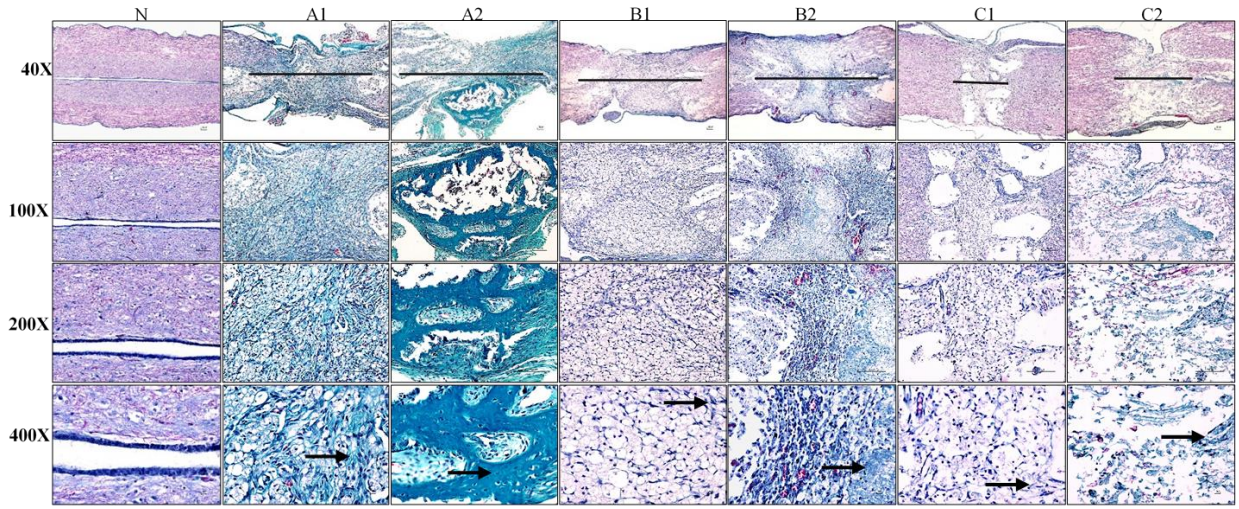

Figure 2:- - Photomicrographs of 5 µm thick Massson's Trichrome stained spinal cord longitudinal sections at 40X, 100X, 200X & 400X showing prominent injury site marked by black lines and comparison of deposited collagen (glial scar central core) marked by the arrow at injury site between all experimental groups. **(N)** Demonstrates normal spinal cord structure with peripheral white matter, central grey matter and central canal in the centre lined by ependymal cells.

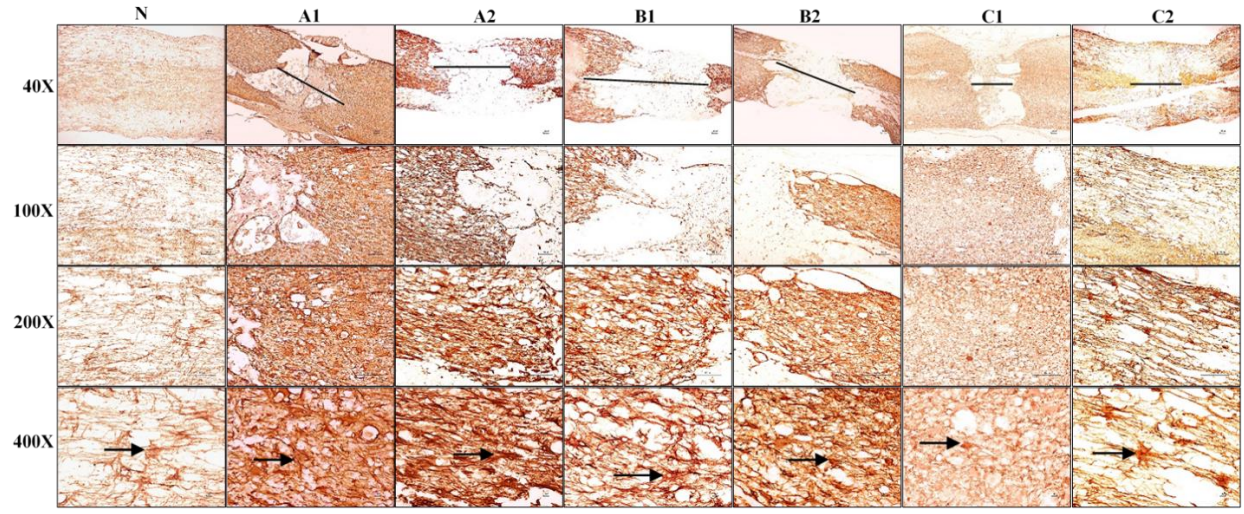

Figure **Error! No text of specified style in document.**: - Photomicrographs of 5 µm thick GFAP antibody stained spinal cord longitudinal sections at 40X, 100X, 200X & 400X showing prominent injury site marked by black lines and comparison of reactive astrocytosis (glial scar outer core) around the injury site marked by arrows between all experimental groups. **(N)** Demonstrates normal spinal cord structure with peripheral white matter, central grey matter and normal astrocytes.

Discussion

As a universal problem, the treatment of spinal cord injury has constantly been the point of attention for researchers. Spinal cord injury consists of primary injury and secondary injury, in which prevention and reduction of secondary injury is the main aim of treatment. The glial scar is one of the major products of secondary injury and is considered to be one of the main hindrances in the growth of damaged axons which leads to poor recovery and outcomes after spinal cord injury. In our present study, we used pirfenidone for the first time to reduce glial scar formation after spinal cord injury by decreasing oxidative stress, inflammation and fibrosis. Thus, it seems applicable to use pirfenidone as an anti-inflammatory and anti-fibrotic agent to improve functional recovery after spinal cord injury. We haven't found any study in the literature in which glial scar was quantified and compared in pirfenidone and non-pirfenidone-treated subjects after compression spinal cord injury. So we compared our study with recent studies where pirfenidone was tested as an anti-fibrotic and anti-inflammatory therapy in CNS.

BBB score results of our present study substantiate the results of a recent study conducted by Zhang B (2021) who reported improvement in BBB scores and inclined plate test scores of pirfenidone-treated rats as compared to the control group, in just 7 days after a moderate contusion spinal cord injury was induced by weight drop method [30]. Our current study is in agreement with the study conducted by Ji J et al (2022) in which they assessed the anti-fibrotic effect of Pirfenidone loaded Hyaluronic Acid Methacryloyl hydrogel (HAMA) in preventing epidural fibrosis (adhesions) after

Laminectomy in rats. They detected an extreme decrease in the collagen quantity and fibroblast number in the 120-mg/ml pirfenidone-HAMA treated group compared to the control laminectomy group, 8 weeks after laminectomy [31]. Our study results are supported by a study conducted by Shi K et al (2019) in which they evaluated the anti-fibrotic role of pirfenidone by inhibition of TGF-β1 induced epidural fibroblast proliferation in rat laminectomy model. They detected an intense reduction in the collagen density and fibroblasts number in 40-mg/ml pirfenidone treated and 80-mg/ml pirfenidone-treated groups compared to the 10-mg/ml pirfenidone treated group and control group, on 28 post laminectomy day [32]. The same results are demonstrated in the study conducted by Güvenç Y et al (2018) in which anti-fibrotic and anti-inflammatory effects of pirfenidone by inhibiting IL-1, TNF-α and α-SMA, were demonstrated in attenuating epidural fibrosis after laminectomy. Locally applied 25 mg/kg/day pirfenidone group showed low grades of fibrosis as compared to the SCI group, evaluated by Masson's Trichrome stain [33].

Conclusion

The glial scar formed following spinal cord injury is considered to be one of the main factors that make hindrance and unfavourable conditions for axonal growth and neurological recovery. Meningeal fibroblasts activation and migration towards the injury site play role in establishing a central fibrous core of the glial scar. Furthermore, astrocytes become hypertrophic and proliferate, forming the outer core of the glial scar. These migrated meningeal fibroblasts and proliferated hypertrophic reactive astrocytes are mainly responsible for secreting collagen fibres and other fibrotic components like extracellular matrix in the glial scar which limits axonal regeneration after spinal cord injury.

Our results concluded that pirfenidone has anti-fibrotic and anti-inflammatory inflammatory effects on reducing glial scar formation following spinal cord injury by inhibiting activation and migration of meningeal fibroblasts, and proliferation & reactivation of astrocytes. Thus providing space and a favorable environment for axonal regeneration which leads to better neurological recovery.

Author contribution: All the authors contributed equally.
Conflict of interest: The authors declare no competing interest.
Funding: Any agency did not fund the current research work.
Submission declaration: The work described above has not been published formerly and it is not under consideration for publication somewhere else. All authors approved the publication and if accepted in this journal, will not be published elsewhere.
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References

1. Abu-Baker NN, Al-Zyoud NmH, Alshraifeen A. Quality of life and self-care ability among individuals with spinal cord injury. Clinical Nursing Research. 2021;30(6):883-91.

2. Noonan VK, Dvorak MF, Fehlings MG. Epidemiology of traumatic and nontraumatic spinal cord injury. Future Medicine; 2013.
3. Oyinbo CA. Secondary injury mechanisms in traumatic spinal cord injury: a nugget of this multiply cascade. Acta Neurobiol Exp (Wars). 2011;71(2):281-99.
4. Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, et al. Acute spinal cord injury, part I: pathophysiologic mechanisms. Clinical neuropharmacology. 2001;24(5):254-64.
5. Sekhon LH, Fehlings MG. Epidemiology, demographics, and pathophysiology of acute spinal cord injury. Spine. 2001;26(24S):S2-S12.
6. Chen K, Deng S, Lu H, Zheng Y, Yang G, Kim D, et al. RNA-seq characterization of spinal cord injury transcriptome in acute/subacute phases: a resource for understanding the pathology at the systems level. PloS one. 2013;8(8):e72567.
7. Forgione N, Fehlings MG. Novel insights into pathophysiology and emerging therapeutic opportunities. Future Medicine; 2013.
8. Vogelaar CF, König B, Krafft S, Estrada V, Brazda N, Ziegler B, et al. Pharmacological suppression of CNS scarring by deferoxamine reduces lesion volume and increases regeneration in an in vitro model for astroglial-fibrotic scarring and in rat spinal cord injury in vivo. PLoS One. 2015;10(7):e0134371.
9. Soderblom C, Luo X, Blumenthal E, Bray E, Lyapichev K, Ramos J, et al. Perivascular fibroblasts form the fibrotic scar after contusive spinal cord injury. Journal of Neuroscience. 2013;33(34):13882-7.
10. Kong X, Gao J. Macrophage polarization: a key event in the secondary phase of acute spinal cord injury. Journal of cellular and molecular medicine. 2017;21(5):941-54.
11. Macías-Barragán J, Sandoval-Rodríguez A, Navarro-Partida J, Armendáriz-Borunda J. The multifaceted role of pirfenidone and its novel targets. Fibrogenesis & tissue repair. 2010;3(1):1-11.
12. Seifirad S. Pirfenidone: A novel hypothetical treatment for COVID-19. Medical hypotheses. 2020;144:110005.
13. Cain W, Stuart R, Lefkowitz D, Starnes J, Margolin S, Lefkowitz S. Inhibition of tumor necrosis factor and subsequent endotoxin shock by pirfenidone. International journal of immunopharmacology. 1998;20(12):685-95.
14. Braim AEP, MacDonald MH, Bruss ML, Grattendick KJ, Giri SN, Margolin SB. Effects of intravenous administration of pirfenidone on horses with experimentally induced endotoxemia. American journal of veterinary research. 2009;70(8):1031-42.
15. Shi Q, Liu X, Bai Y, Cui C, Li J, Li Y, et al. In vitro effects of pirfenidone on cardiac fibroblasts: proliferation, myofibroblast differentiation, migration and cytokine secretion. PloS one. 2011;6(11):e28134.
16. Hale ML, Margolin SB, Krakauer T, Roy CJ, Stiles BG. Pirfenidone blocks the in vitro and in vivo effects of staphylococcal enterotoxin B. Infection and immunity. 2002;70(6):2989-94.
17. Schaefer C, Ruhrmund D, Pan L, Seiwert S, Kossen K. Antifibrotic activities of pirfenidone in animal models. European Respiratory Review. 2011;20(120):85-97.
18. Gokce EC, Kahveci R, Atanur OM, Gürer B, Aksoy N, Gokce A, et al. Neuroprotective effects of Ganoderma lucidum polysaccharides against traumatic spinal cord injury in rats. Injury. 2015;46(11):2146-55.
19. Curtin LI, Grakowsky JA, Suarez M, Thompson AC, DiPirro JM, Martin LB, et al. Evaluation of buprenorphine in a postoperative pain model in rats. Comparative medicine. 2009;59(1):60-71.

20. Sliwinski C, Nees TA, Puttagunta R, Weidner N, Blesch A. Sensorimotor activity partially ameliorates pain and reduces nociceptive fiber density in the chronically injured spinal cord. Journal of neurotrauma. 2018;35(18):2222-38.
21. Emamhadi M, Soltani B, Babaei P, Mashhadinezhad H, Ghadarjani S. Influence of sexuality in functional recovery after spinal cord injury in rats. Archives of Bone and Joint Surgery. 2016;4(1):56.
22. Ferrero SL, Brady TD, Dugan VP, Armstrong JE, Hubscher CH, Johnson RD. Effects of lateral funiculus sparing, spinal lesion level, and gender on recovery of bladder voiding reflexes and hematuria in rats. Journal of neurotrauma. 2015;32(3):200-8.
23. de Barros Filho TEP, Molina AEIS. Analysis of the sensitivity and reproducibility of the Basso, Beattie, Bresnahan (BBB) scale in Wistar rats. Clinics. 2008;63(1):103-8.
24. Guidlines A. AVMA Guidelines for the Euthanasia of Animals USA2020 [2020:[Available from: https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf.
25. Support BR. Euthanasia of Rodents: BU IACUC; 2021 [Available from: https://www.bu.edu/researchsupport/compliance/animal-care/working-with-animals/euthanasia/euthanasia-of-rodents/.
26. S. Kim Suvarna CL, John D. Bancroft. Bancroft's THEORY and PRACTICE of HISTOLOGICAL TECHNIQUES. Eighth Edition ed. USA: Elsevier Limited; 2019. 30 p.
27. IHCWORLD. Life Science Products & Services IHC WORLD [Available from: http://www.ihcworld.com/_protocols/special_stains/masson_trichrome.htm.
28. Hofman F. Immunohistochemistry. Current protocols in immunology. 2002;49(1):21.4. 1-.4. 3.
29. Kim S-W, Roh J, Park C-S. Immunohistochemistry for pathologists: protocols, pitfalls, and tips. Journal of pathology and translational medicine. 2016;50(6):411-8.
30. 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;37:102420.
31. Ji J, Cheng J, Chen C, Lu Y, Chen X, Zhang F. Pirfenidone-loaded hyaluronic acid methacryloyl hydrogel for preventing epidural adhesions after laminectomy. Drug Delivery and Translational Research. 2022:1-12.
32. Shi K, Wang F, Xia J, Zuo B, Wang Z, Cao X. Pirfenidone inhibits epidural scar fibroblast proliferation and differentiation by regulating TGF-β1-induced Smad-dependent and-independent pathways. American journal of translational research. 2019;11(3):1593.
33. GÜVENÇ Y, BİLLUR D, BAYRAM P, Öztürk Y, YAMAN M, AYDIN S, et al. PIRFENIDONE ATTENUE EPIDURAL FIBROSIS IN RATS BY SUPPRESSING TNF-α, IL-1, AND α-SMA. the journal of spinal surgery. 2018;29(3).