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Revitalizing Lower Motor Neurons Function: Role of Pirfenidone in Recovery of Post-Compression Spinal Cord Injury

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Abstract

Background: Spinal cord compression injury can cause severe motor deficits and significantly reduce quality of life and effective motor recovery therapies are limited. This article examines the latest research on pirfenidone potential to enhance motor recovery in SCCI and transform therapeutic approaches.

Objective: The aim of study was to establish whether pirfenidone delivered intraperitoneally can improve lower motor neuron activity in rats following compression injury to spinal cord or otherwise.

Material and Method: This experimental study was carried out at the Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan, from September 2021 to December 2022. Induction of injury to T7 spinal cord level was performed by 70gm force aneurysm clip on rats. The group "A" was given a placebo daily, the group "B" was given 200 mg/kg/day daily dose of pirfenidone, and the group "C" was given a daily dose of pirfenidone 500 mg/kg/day. Using 14 & 28 day experimental durations, sub-grouping of all 3 groups was done into groups 1 & 2 (each sub-group having 5 rats). On the final day of the experiment, BBB scoring was conducted to assess lower motor neuron activity in the hind limbs of all rats.

Results: The BBB (Basso, Beattie, Brenham) scores were statistically different between and within groups. The spinal cord injury (SCI) groups that received pirfenidone treatment exhibited higher BBB scores in comparison to the SCI groups that did not receive pirfenidone. The 500 mg/kg/day and a 28-day period of pirfenidone were more effective in improving motor recovery after spinal cord injury than 200 mg/kg/day for a 14-day period.

Conclusion: Pirfenidone is likely to enhance motor functions, contributing to improved functional neurological recovery following spinal cord injury.

Keywords: Pirfenidone, Aneurysm Clip Model, Spinal Cord Compression Injury, BBB score

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Introduction:

The overall qualities of life for patients with spinal cord injuries are often worse than one could ever imagine. Traumatic SCI results in substantial primary sensory and motor deficits that usually follow a permanent course, the consequences of which need to be actively managed[1]. There are two pathophysiological stages in spinal cord injury, the primary and secondary stages can take place[2]. Spinal cord injury perturbs the integrity of spinal nervous tissue, thereby inducing vast amount of primary damage as it occurs mechanical compression by incurring an initial trauma[3]. Burst fractures and fracture-dislocation injuries are some of the most common examples of primary compression injuries[4]. Several minutes after the initial primary injury, a secondary injury begins and may last for several weeks to months. It induces secondary injury manifested by increased levels of neuro-inflammation and oxidative stress, edema, ischemia, the formation of cyst cavities and astrocytic glial scarring[5]. Both primary and secondary SCIs are also classically grouped by development stages time line as, immediate/instantaneous, acute, sub-acute (intermediate) and chronic[6]. This is accompanied by an infiltration of inflammatory phagocytes and macrophages, followed by meningeal and perivascular growth of fibroblasts along with extensive reactive

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astrocytosis that drags the period of sub-acute damage for up to two weeks. In addition, oxidative stress further aggravates neuron demyelination as well as the apoptosis at the intermediate phase. This will result in the formation of glial scars in the next stage, due to unbalanced rearrangement of hypertrophic astrocytic processes around the lesion and collagen deposition by fibroblasts and reactive astrocytes involved in glial scar inhibits axonal repair and regeneration. The use of the above-mentioned products in the lesion site prevents axonal sprouting and regeneration, thereby improving neurological rehabilitation and functional recovery[7-9].

Initially researched in helminths and fevers, pirfenidone (PFD) was first developed. PFD is incredibly small and can zip in and out of the blood-brain-barrier (BBB) leading to it be systemic and reach most organs when taken orally[10-11]. Pirfenidone has antifibrotic, as well as antioxidant and anti-inflammatory properties. It inhibits fibroblast growth, diminishes production of fibrosis-associated proteins and cytokines, and enhances accumulation/retention of extracellular matrix in response to key growth factors including transforming growth factor (TGF) and platelet-derived growth factor[12]. Various experiments showed that it has inhibitory effects in anti- cytokine factor such as TNF, interleukins and many inflammatory cytokines[13]. In this study, therefore, we sought to explore the potential of pirfenidone on enhancing lower motor neuron activity after compression injury to spinal cord.

Materials and Methods

This study was carried-out at the Institute of Basic Medical Sciences, Khyber Medical University, Peshawar Pakistan after obtaining approval by the institutional ethical review board (Reference number Dir/KMU-EB/RP/000768). The study included 30 healthy male Sprague Dawley rats weighing 250-300 g at the age of 3–4 months. Exclusion of female rats may complicate breeding efficiencies. The number of animals used in the study was calculated by the resource equation method and based on the 3R's principle (Replacement, Reduction and Refinement). The rats were kept in a temperature-controlled environment (22–25°C), with relative humidity and a 12 h light/dark cycle. All of the experiments were in accordance with National Research Council's Guide for the care and use of laboratory animals.

Grouping of 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 (5 in each sub-group). Groups A1 and A2 received DMSO (dimethyl sulfoxide) intraperitoneally as a placebo daily. A compression spinal cord injury was induced in group B1 and group B2, and 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 daily administered intraperitoneally in DMSO as solvent[14].

Surgical Procedure:

After anesthesia had been induced, an incision was made carefully along the back of rat at the level about T7 vertebral. The spinous processes of T7 vertebrae were resected and carried out a total laminectomy to take away the dorsal lamina. A 70-g force aneurysm clip was placed in the mid-portion of the exposed T7 spinal cord segment with the meninges still directly intact. After leaving it for one minute, the clip was taken out and wound was layered back. Proper antibiotics and analgesics were used to prevent infection, reduce post-operative pain and swelling[15].

Motor Assessment:

The rats of the groups A1, B1 and C1 were submitted to motor tests on day 15 after beginning of treatment, while those of the A2, B2 and C2 groups on day 29. Motor evaluations were performed using the BBB (Basso, Beattie, Bresnahan) scoring system with grades ranging from 0 to 21 points based on combined efforts of various joint movements. These scores were based on the movement, and coordination of hind and forelimbs, placing of the paws during walking, trunk position during locomotion and tail position.

Transparent fiberglass sheet was used for the sides and part of the top, while all structures were covered by a reinforced one-piece tent structure. The interior was treated with black non-slip paint to treat the floor for better visibility during motor activity. A box size of 80 x 80 x 30 cm was used and three digital cameras (as shown in Figure. 1) were mounted outside the box at different angles to deliver videos for validation. Every rat was positioned inside the compartment and inspired to relocate for 4 minutes. The synchronized movements of the joints in rats were meticulously observed and documented. Each rat was scored by two independent and blinded assessors using a movement scoring scale of 0–21 based upon their spontaneous utilization of the previously trained movements.[16].

Figure 1: Photographs of BBB scoring performance for assessing motor reflexes in hind limbs of rats from different groups. (A) Rat bearing body weight with occasional plantar stepping and no coordination of fore limbs with hind limbs. (B) Constant plantar stepping of rat with constant coordination of all limbs and frequent toes movement; hind limb paw position is parallel to the

body tail position downward occasionally or constantly (C) Rat not bearing body weight with plantar sustenance of hind limb paw in the support stage when not moving with curved toes. (D) Rat having rotated paws, wide range of moments in hind limbs, plantar sustenance of the paw in the hind limbs without weight bearing when not moving. (E) Rat dragging its hind limbs with insignificant restricted movement in hip & knee joint. (F) Rat showing widespread movement of two joints with limited movement of third joint in the hind limbs.

Data was analyzed by SPSS 22 and mean and standard deviation calculated for descriptive statistics. Group comparisons were performed with the Kruskal-Wallis test for between-group variation and Mann-Whitney U tests to inspect within-group deviation. Statistical significance was defined for a p-value less than 0.05.

Results:

Figure 2 shows the mean BBB scores for all groups. BBB scores differed significantly between the groups A1 & A2, B1 & B2, and C1 & C2 by respective p-values of 0.008, 0.009, and 0.001 as indicated in Figure 2-A. P-value of 0.002 represents the high significance of the difference between A1, B1, and C1 in BBB scores. A2, B2 and C2 also demonstrated high significance with a difference of 0.004 between the BBB scores as shown in Figure 2-B.

According to these 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 were more effective in improving motor recovery after spinal cord injury than 200 mg/kg/day for a 14-day period.

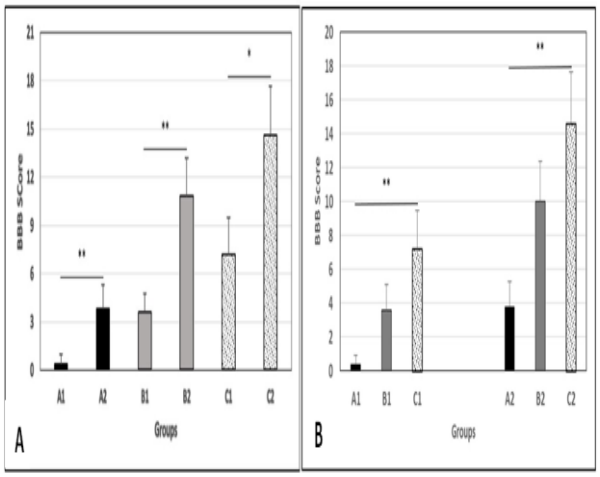

Figure 2:(2-A) Comparing the average BBB scores within each group. (2-B) Assessing the mean BBB scores between the groups. Group "A1" refers to the non-pirfenidone treated group with a 14-day experimental duration. Group "A2" denotes the non-pirfenidone treated group with a 28-day experimental duration. Group "B1" represents the 200 mg/kg/day pirfenidone treated group with a 14-day experimental duration. Group "B2" signifies the 200 mg/kg/day pirfenidone treated group with a 28-day experimental duration. Group "C1" pertains to the 500 mg/kg/day pirfenidone treated group with a 14-day experimental duration. Group "C2" signifies the 500 mg/kg/day pirfenidone treated group with a 28-day experimental duration. The symbol "┬" denotes standard deviation (SDEV). Statistical analysis involved the Mann Whitney U test and Kruskal Wallis test, revealing p-values <0.05 both within and between the groups.

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 is 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 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 of spinal cord injury [18]. These results are comparable and similar to our present study findings 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:

It is 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.

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