Can Enforced Behavioral Activity in Spinal Cord Injured Rats be considered as Rehabilitation Process to Accentuate Tacrolimus Treated Recovery? A nursing Care Perspective

Mohammad Ahmad\(^1\) and Ahmad Abo Shaiqah\(^2\)

\(^1\)Department of Medical Surgical Nursing, College of Nursing, King Saud University, Riyadh, Saudi Arabia
\(^2\)Department of Nursing Administration and Education, College of Nursing, King Saud University, Riyadh, Saudi Arabia

**Abstract**

Animal models for spinal cord injury (SCI) help in developing effective treatment modalities. The aim of the present study is to develop suitable rehabilitative intervention besides therapeutical agents for a better functional recovery. Furthermore, the research intends to bring awareness among the nurses for caring SCI patients and utilizing their passion of caring abilities in nursing research also. Young adult male rats were subjected to spinal trauma by compression method of the exposed spinal cord. Animals were allocated to five groups with eight animals in each, viz. Group 1 as normal uninjured control; group 2 as sham control with laminectomy but no spinal injury; group 3 as SCI group with laminectomy and spinal injury; group 4 as SCI treated group A that were same as group 3 but were treated with a daily injection of Tacrolimus (1 mg/kg) for 29 days and subjected to BBB behavioral test in which the hind limb function was scored from 0 (complete paralysis or paraplegia) to 21 (complete mobility), every alternate days in a “Gait Performance Tunnel” (GPT); group 5 as SCI treated group B that were same as group 4 except that the animals were also subjected to a daily enforced extra 5 walks as exercise in GPT. Although the drug had an attenuating effect on SCI in both treated groups A and B the recovery in Group-B was significantly (p<0.001) greater than Group-A. It is concluded that if the SCI animals are subjected to enforced daily behavioral exercises in addition to drug treatment, it can improve functional recovery at a faster rate and can be considered as a rehabilitative activity to accentuate therapeutic treatments. Furthermore, the present study can be a source of inspiration for the nurses to develop their nursing skills and research abilities.

**Keywords:** Spinal cord injury; Tacrolimus; BBB scoring; Rehabilitation; Nursing care

**Introduction**

Experimental studies using suitable animal models for spinal cord injury (SCI) help to simulate clinical conditions as observed in humans and play an important role to understand the pathophysiology of the disease and to develop effective treatment modalities. To date no satisfactory drug treatment or other methods of interventions like physiotherapeutically related rehabilitation process have been designed to repair the traumatically injured spinal cord in humans. The aim of the present study is to understand the behavioral recovery that often occurs following the initial primary injury, and to develop suitable rehabilitative intervention including pharmacological agent to enhance improved sensory and motor function. Furthermore, this research intends to bring awareness among the nurses working in general wards, specialized wards, and occupational health set-ups, to understand the need to show their passion for SCI patient's care by exploring and learning standardized and improvised rehabilitation methods to manage and practice effective nursing caring skills for SCI patients.

Tacrolimus (also known as FK506), a macrolide lactone antibiotic, was introduced as an immunosuppressive agent [1] with virtually no side effects [2]. Tacrolimus, a potent calcineurin inhibitor exhibits neuroprotection actions in several experimental models of central nervous system trauma, including stroke and improved neurological recovery following peripheral and spinal cord injuries [2-6]. However, some side effects from oral and intravenous administration of Tacrolimus in clinical case studies have been reported which include nephrotoxicity [7], lung damage [8], various neuropsychiatric problems, neurotoxic effects such as akinetic mutism and catatonic mutism [9,10]. On the contrary, in experimental studies, Tacrolimus improves the functional outcome of spinal cord injury [5,11,12] and has an in vivo neurotrophic action whereby it enhances the rate of axon regeneration leading to more rapid neurological recovery [13-16].

Thus, the present study was designed to investigate the neuroprotective effects of Tacrolimus on behavioral recovery from SCI. Furthermore, from the perspective of educating the nursing community from a rehabilitative awareness point of view, the present experimental study was designed to asss to if the repetitive reinforcement of SCI subjects to extra sequential behavioral activities in addition to pharmacological treatment do accentuate the rehabilitation process for a better functional recovery from SCI or not?

**Materials and Methods**

**Animals**

Young adult male Sprague-Dawley rats, weighing 250-280 g. bred, reared and housed under controlled conditions (diurnal 12 hour light-dark cycle, temperature 22 ± 1°C, humidity 50-60%, free access to food.
and water) in the animal facility of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, were used in the present study. All care was taken to minimize animal stress and suffering. Moreover, all animal practices and animal study protocols were approved by the Research and Ethics Committee of King Saud University, Riyadh, Saudi Arabia, for the humane care of animals.

Spinal cord injury (SCI)

Rats were anesthetized with chloral hydrate (450 mg/kg), and were subjected to spinal trauma by the modified method of Nystrom and Berglund [17]. After shaving the back of the animal, a longitudinal incision was made on the midline of the back, exposing the paravertebral muscles. Laminectomy was performed under surgical operating binocular microscope, at T 7-8 level leaving the dura intact. SCI was produced by placing a metallic rectangular plate (2.5 x 5.0 mm) loaded with a total weight of 35 g for 5 min, over the exposed extradural area of the spinal cord for compression. The wound was closed in layers through aseptic surgical stitching and animals were allowed to recover from anesthesia by placing them on a warm heating pad maintained at 37 ± 1°C. All animals were given intramuscular injections of gentamycin at a dose of 3 mg/kg for 3 days after surgery. The animals’ bladders were manually pressed twice a day to avoid urinary complications until the rats were able to regain normal bladder functioning. Sham injured animals were only subjected to laminectomy with the same surgical procedures without any compression.

Experimental groups

Rats were randomly allocated to the following five groups with eight animals in each group:
1. Normal control group; without any laminectomy or compression injury.
2. Sham group; with laminectomy only but no spinal compression injury.
3. SCI control group; with laminectomy surgery and spinal compression injury.
4. SCI treated groups A and B; these were same as SCI control groups except that SCI Treated group-A and group-B were used to study the effect of Tacrolimus (1 mg/kg) treatment for the recovery from SCI using behavioral parameter of BBB scoring every alternate day as described below. The dose of this drug was selected on the basis of our pilot screening at low, medium and high doses (0.5, 1.0 and 1.5 mg/kg, respectively). The best effective drug dose of Tacrolimus (1 mg/kg) was selected based on those pilot studies. The drug was administered orally in the morning session always. The first dose of the drug was administered one hour after SCI and thereafter, daily for three weeks.

Behavioral analysis

The behavioral motor functions in the form of BBB scorings were observed in the evening session and were assessed in a blind manner. The scores of each test were evaluated the next day after injury and every alternate day for 29 days after SCI for each animal.

BBB scoring: Hind limb motor function (including hind limb reflexes and coordinated use of hind limbs) was assessed using the Basso-Beatie-Breshnahan (BBB) locomotor rating scores [18]. The method for scoring this BBB rating was modified in a sense that instead of placing the individual rats in an open field for the evaluation of the hind limb motor behavior, the animals were allowed to travel through a “Gait Performance Tunnel” (GPT). This innovative GPT consisted of a narrow tunnel, constructed from a wooden block of size (180 × 10 × 5 cm) with side-walls made of clear perspex glass (180 × 18 cm²), so that the animal movement was clearly visible from the side walls to the blinded observer and the score was assessed carefully for the rehabilitative coordinated movement of the hind limbs and placement of the hind paws. The GPT was placed at a height of 30 cm on the working table. The animal was allowed to enter at one end and travel through up to the other end. Soft bedding was placed under the other end of the GPT in order to avoid any injury in case of a fall from the GPT. No time was fixed for the walk in the GPT. The observer was able to assess the movements of hind limbs and placement of hind paws by the animals easily, through the Perspex glass sheet of the GPT. Hind limb function was scored from 0 (complete paralysis or paraplegia) to 21 (complete mobility).

SCI treated group-A and group-B were subjected to similar experimental processes to observe the behavioral parameter of BBB scoring every alternate day, except that the animals of group-B were further repeatedly subjected to a daily additional 5 times enforced extra walking on the GPT with an interval of 5 min rest between the walks. Thus, the animals of group-A after completing the BBB test on alternate days, were left in their home cages with no further disturbance. Whereas, animals of group-B, after BBB scorings, were subjected to an additional daily enforced five extra walks through the GPT. After completing 5 additional extra walks, the animals were left in their home cages in a manner similar to group-A.

Statistical analysis

The data from the experimental SCI group passing the normality test (p>0.10) were compared to the SCI uninjured control group, whereas the data of drug-treated groups were compared with the experimental SCI group using ANOVA with post-hoc testing using Tukey-Kramer Multiple Comparison Tests or Student-Newman-Keuls Multiple Comparison Tests. All results were expressed as means ± SEM and significance was defined as p<0.05 for the test.

Results

The results indicated that treatment with the drug FK506 induced recovery from SCI with respect to time. Although the drug had an attenuating effect on SCI in both treated groups A and B as compared to the SCI only control group, the effectiveness of this drug on the behavioral recovery in treated Group-B was significantly (p<0.001) greater than in treated Group-A from SCI (Figure 1). The sham group showed minimal alterations in behavioral activities and attained similar scoring levels to the naïve control groups within a few days, indicating no contusion damage in the spinal cord. Although the behavioral recovery from SCI (in the SCI only group) in 29 days was lesser significant [F(1)=26.18, p<0.01] compared to the naïve control, the drug treated SCI groups A and B showed better and improved recovery in BBB behavioral scorings compared to the SCI only control group, and the drug treated groups A and B were effective in the order of FK506-groupB>FK506-groupA (F=11.35 and F=6.72, df=2, p<0.001 and p<0.01, respectively) as shown in Figure 1.
Conclusion

It is evidently concluded from this study that if the SCI animals are subjected to enforced daily repetitive behavioral exercises (in the present study as enforced walk in the GPT), it can improve functional restoration at a faster pace from SCI and it can be considered as a rehabilitative process to accentuate therapeutic treatments (Tacrolimus in present study). Such interventions with physiotherapeutically related rehabilitation process can also be helpful to repair the traumatically injured spinal cord in humans. From the nursing care perspective, the present study can also serve as a model research activity for the general nurses and/or the specialized nurses for an improved rehabilitation service to the affected SCI patients for better healthcare and wellbeing. Furthermore, the emphasis of increased clinical research nowadays has led to a need to innovate, improve and standardize education on research opportunity provided to nurses also. Thus, the ability to show compassion in clinical practice is a crucial nursing skill and the present study can be a source of inspiration for the inextricable link for bringing change in the delivery of compassionate care by the healthcare nurses. However, more detailed studies are required to confirm this presumption.

Acknowledgement

The author is thankful to the Deanship of Scientific Research, College of Nursing Research Center at King Saud University for funding this research.

References

1. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, et al. (1989) FK 506 for liver, kidney and pancreas transplantation. Lancet 2: 1000-1004.
2. Sosa I, Reyes O, Kullfer DP (2005) Immunosuppressants: Neuroprotection and promoting neurological recovery following peripheral nerve and spinal cord lesions. Exp Neurol 195: 7-15.
3. Bochelen DMR, Sauter A (1999) Calcineurin inhibitors FK506 and SDZASM981 alleviate the outcome of focal cerebral ischemic/reperfusion injury. J Pharmacol Exp Ther 288: 653-659.
4. Butler SP, Henshall DC, Teramura Y, Iwasaki K, Sharkey J (1997) Neuroprotective actions of FK506 in experimental stroke: In vivo evidence against an antixcitototic mechanism. J Neurosci 17: 6939-6946.
5. Madsen JR, McDonald P, Irwin N, Goldberg DE, Yao GL, et al. (1998) Tacrolimus (FK506) increases neuronal expression of GAP-43 and improves functional recovery after spinal cord injury in rats. Exp Neurol 154: 673-683.
6. Saganová K, Gálik J, BlaÅ¡ko J, Korimová A, RaÄeková E, et al. (2012) Immunosuppressant FK506: Focusing on neuroprotective effects following brain and spinal cord injury. Life Sci 91: 77-82.
7. Naesens M, Kuypers DR, Sarwal M (2009) Calcineurin inhibitor nephrotoxicity. Clin J Am Soc Nephrol 4: 481-508.
8. Miwa Y, Izsoki T, Wakabayashi K, Odai T, Matsuwana M, et al. (2008) Tacrolimus-induced lung injury in a rheumatoid arthritis patient with interstitial pneumonitis. Mod Rheumatol 18: 208–211.
9. O’Donnell MM, Williams JP, Weinrieb R, Denysenko L (2007) Catatonic mutism after liver transplant rapidly reversed with lorazepam. Gen Hosp Psychiatry 29: 280-281.
10. Chopra A, Das P, Rai A, Kuppuswamy PS, Li X, et al. (2012) Catatonia as a manifestation of tacrolimus-induced neurotoxicity in organ transplant patients: A case series. Gen Hosp Psychiatry 34: 209.
11. Yamaji T, Yamazaki S, Li J, Price RD, Matsuwa N (2008) FK1706, a novel non-immunosuppressant neurophilin ligand, ameliorates motor dysfunction following spinal cord injury through its neurodegenerative effects. Eur J Pharmacol 591: 147-152.
12. Zhang J, Zhang A, Sun Y, Cao X, Zhang N (2009) Treatment with immunosuppressants FTY720 and tacrolimus promotes functional recovery after spinal cord injury in rats. Tohoku J Exp Med 219: 295-302.

13. Gordon T, Sulaiman O, Boyd JG (2003) Experimental strategies to promote functional recovery after peripheral nerve injuries. J Peripher Nerv Syst 8: 236–250.

14. Navarro X, Udina E, Ceballos D, Gold BG (2001) Effects of FK506 on nerve regeneration and reinnervation after graft or tube repair of long nerve gaps. Muscle Nerve 24: 905-915.

15. Sulaiman OA, Voda J, Gold BG, Gordon T (2002) FK506 increases peripheral nerve regeneration after chronic axotomy but not after chronic Schwann cell denervation. Exp Neurol 175: 127–137.

16. Udina E, Rodrigues FJ, Verdu E, Espejo M, Gold BG, et al. (2004) FK506 enhances regeneration of axons across long peripheral nerve gaps repaired with collagen guides seeded with allogenic Schwann cells. Glia 4: 120–129.

17. Nyström B, Berglund JE (1988) Spinal cord restitution following compression injuries in rats. Acta Neurol Scand 78: 467-472.

18. Basso DM1, Beattie MS, Bresnahan JC (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 12: 1-21.

19. Wells JE, Hurlbert RJ, Fehlings MG, Yong VW (2003) Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice. Brain 126: 1628–1637.