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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*

[*]**Corresponding author:** Ahmad M, Department of Medical Surgical Nursing, College of Nursing, King Saud University, Riyadh, Saudi Arabia, Tel: +966-505195887; E-mail: mbadshah@ksu.edu.sa

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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, this 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 lactane 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 assess as to if the repetitive enforcement 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 × 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 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 cm^2), 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 naive 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.

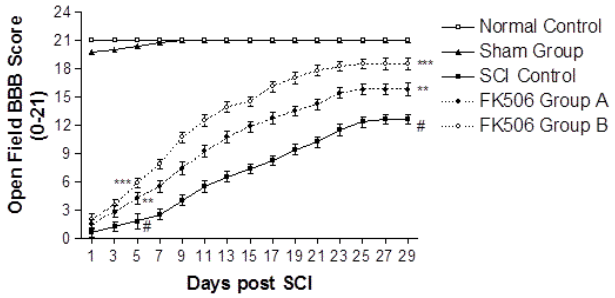

Figure 1: Effect of FK506 on gait performance tunnel (GPT) behavioral motor performance activities (BBB Score) of hind limbs of rats subjected to spinal cord injury (SCI). The graph shows the comparative functional recovery from SCI over a period of 29 days. Animals were treated with the drugs daily after SCI for 3 weeks. FK506 Group A is (1 mg/kg with no enforced walk) and FK506 Group B is (1 mg/kg with daily enforced walk). The groups are effective in the order Group B>Group A; # shows the SCI group significantly ($p<0.001$) different from the SCI uninjured control group; ** and *** represent the SCI FK506 treated groups A and B significantly different at $p<0.01$ and $p<0.001$, respectively, compared to the SCI group by ANOVA with post-hoc testing using Tukey-Kramer or Student-Newman-Keuls Multiple Comparison Tests.

Discussion

In this study, recovery from SCI in all control, sham and treated groups A and B were assessed from overall comparisons of all scoring results from behavioral parameters of BBB score (Figure 1). The SCI animals exhibited severe deficits in hind limb function and moved using only the forelimbs, dragging the paraplegic hind limbs. Because the functional recovery of both treated groups A and B differentiated significantly from the SCI controls from the earliest time point studied (3 days), the mechanism of the drug under study is likely due to neuroprotection rather than tissue regeneration, as suggested earlier [19]. Our results demonstrate that treatment with Tacrolimus in treated groups A and B when administered systematically after SCI, resulted in the significantly recovery of functional deficits starting at 1-week post injury. The treated groups recovered hind limb reflexes more rapidly, and a higher percentage of these rats regained responses comparable with those of injured untreated control rats. The treated rats also achieved a faster and greater degree of recovery of coordinated use of their hind limbs in maintaining their body position from 14 to 28 days (Figure 1). However, between treated groups A and B the better significant functional recovery was observed in group B where the Tacrolimus treated SCI animals were additionally subjected to daily enforced 5 extra walks on the GPT. Thus, the treated groups A and B showed improved recovery in their BBB behavioral activities scorings in the order of Tacrolimus group B>Tacrolimus group A. This significant difference in the movement behavior between the two groups was possibly because in addition to the therapeutic effects of Tacrolimus, the daily enforced extra walks in group B apparently worked as a potential factor in accentuating the animal's behavioral activities as rehabilitative exercises that ultimately helped them to recover significantly faster than the animals of group A from SCI.

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.

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