Electrical stimulation and umbilical cord stem cell transplantation in murine injured sciatic nerve

Amani Abdelfattah Mohamed Ali Elbaz[1], Nagwan A Sabek[1], Elham H Madny[1], Suzan M Hazzaa[2], Ebtehal M Metwally[2], Eman A Mohamed[1].

[1]*Suez Canal University, Egypt*

[2]*Menoufia University, Egypt*

Abstract

Several approaches have been proposed for peripheral nerve regeneration. We aimed to assess effect of electrical stimulation and Mesenchymal Stem Cells (MSCs) on recovery of rat sciatic nerve. Fifty adult male albino rats weighing 180-250 g were divided into 5 groups: Group-1; sham operated intact nerve. Group-2; crushed nerve control group. Group-3; crushed nerve followed by transplantation of MSCs (3×10^5 cells/rat) once intra-lesion immediately after injury. Group-4; crushed nerve followed by applying electrodes 5 mm proximal to the injured site using a biphasic current pulse (100 µs pulse width, 20 Hz pulse rate, 2 µA amplitude) for 30 minutes. Group-5; crushed nerve followed by combining procedures of previous two groups. Wound closure and post-surgical care followed. MSCs were isolated from human umbilical cord blood by Ficoll-Hypaque density gradient centrifugation, culture of mononuclear cells and selection by CD 105+ve CD34-ve CD45-ve magnetic separation method using MACs separator. Behavioral testing before injury and at fourth and eighth weeks, serum malondialdehyde and total antioxidant capacity at 48 hours then electrophysiological studies measured at 8 weeks. After 2 weeks, gene expression of brain derived neurotrophic factor (BDNF) mRNA in injured tissue was measured by real-time-PCR. Treatment with either ES or MSCs transplantation accelerated regeneration in all parameters over 8 weeks of the study. Combined treatment group did not show superiority compared to the other two sole treated groups except in the BDNF expression value. Using MSCs and electrical stimulation give better outcome for peripheral nerve regeneration. Further investigation of combined electrical stimulation and stem cells is recommended.

Introduction

Injuries to the peripheral nervous system may bring about extensive inabilities because of the interference of axons progression, degeneration of nerve fibers distal to the injury and possible death of axotomized neurons (1). Mesenchymal stem cells (MSC) are type of adult derived stem cells that are emerging as an effective therapeutic approach to a wide range of neural insults since they act as a source of stem-like and progenitor cells. The human umbilical cord blood (HUCB) is a valuable source of cells being available and less immunogenic as compared to other sources of stem cell (2*).*). It was reported that 30 minutes of low-intensity electrical stimulation applied after injury can improve regeneration in crushed rat sciatic nerve (3) (4). The aim of present study is to assess the effects of combination of treatment with MSCs transplantation and electrical stimulation on repair of sciatic nerve crush injury.

Materials & Methods

Study Groups				
G 1 (Control normal)	G 2 (Control injured)	G 3 (Injured Mesenchymal Stem Cells (MSCs) treated)	G4 (Injured Electrical Stimulation treated)	G5 (Injured combined treatment)
Intact Sciatic nerve (sham operated)	ICrushed Sciatic nerve	Crushed sciatic nerve followed by transplantation of MSCs (3×10^5 cells/rat) once intra-lesion immediately after injury **(5)**	Crushed nerve followed by applying electrodes 5 mm proximal to the injured site using a biphasic current pulse (100 µs pulse width, 20 Hz pulse rate, 2 µA amplitude) for 30 minutes.	Crushed nerve followed by combining procedures of previous two groups

At the time of induction of sciatic nerve injury

Injury to the sciatic nerve was done using the standard crush injury method under general anesthesia by pentobarbital sodium (100mg/kg). Complete post operative care was performed to all group **(6)**.

Mesenchymal stem cells were isolated from the human umbilical cord blood using the Ficoll-Hypaque density gradient centrifugation, then culture of mononuclear cells and selection by CD105+veCD34-veCD45-ve magnetic separation method using MACS separator **(7)**.

Behavioral assessment using the Walking Track analysis were performed in all groups once before injury **(8).**

After four weeks from the sciatic nerve injury

Walking track analysis was performed to all groups(using sciatic function index SFI).

After eight weeks from the sciatic nerve injury

Walking track analysis was performed to all groups SFI.

EMG using the Biopack, MP 150 system were done at 8th week post injury**(9)**. Rats were sacrified and in vitro nerve conduction velocity was performed immediately .

Measurement of BDNF mRNA level by RT-PCR tehnique **(10).**

Results

Figure 1: **SFI values at 4th and 8th week in the study groups.**

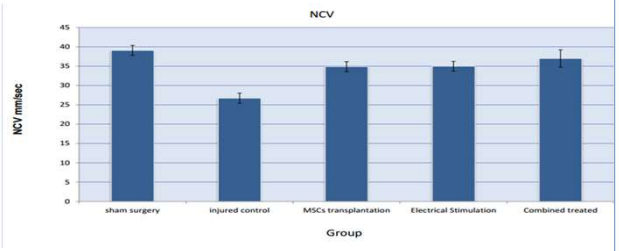

Fig.2 : **Nerve conduction velocity values in mm/s in the study groups**

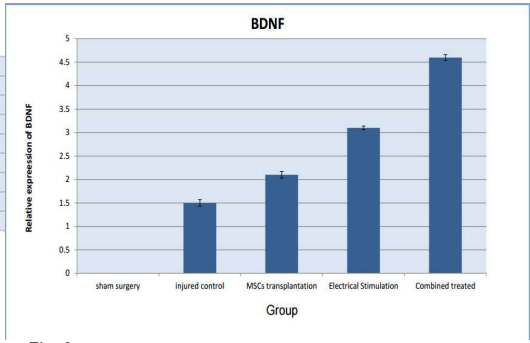

Fig. 3: **BDNF mRNA levels in the study groups.**

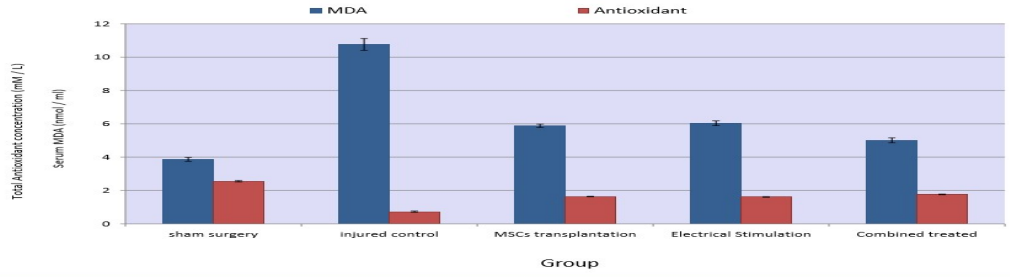

Fig.4 : **oxidative stress enzymes in the study groups.**

Conclusion

Treatment with either ES or MSCs transplantation accelerated regeneration in all parameters over 8 weeks of the study. Combined treatment group did not show superiority compared to the other two sole treated groups except in the BDNF expression value. Using MSCs and electrical stimulation give better outcome for peripheral nerve regeneration. Further investigation of combined electrical stimulation and stem cells is recommended.

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