The Potential Role of Erythropoietin versus Human Umbilical Cord Blood Stem Cell Therapy Following Sciatic Nerve Injury in Rats.

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Abstract

Injuries to peripheral nerves result in partial or total loss of motor, sensory and autonomic functions. We aimed to evaluate the neuroprotective effect of the sole or combined treatment with erythropoietin (EPO) and human umbilical cord blood mesenchymal stem cells (MSCs) transplantation in sciatic nerve injury. Sixty adult male albino rats weighing 200 gm were divided equally into 5 groups: control intact (sham operated) group and four other injured groups: control, erythropoietin treated (5000 U/kg) intraperitoneal four after injury, MSCs treated (2.5×10^4 cells/µl) grafted intralesional immediately after injury and combined EPO and MSCs with same previous doses treated group. 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. Assessment was done functionally by walking track analysis using sciatic function index (SFI) at 4th and 8th weeks post injury, electrophysiologically by electromyography and nerve conduction velocity (NCV) using the Biopack, MP 150 system were done at 8th week post injury. Also, gene expression in injured nerve of brain derived neurotrophic factor (BDNF) mRNA by real time PCR technique was done. 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 groups. The study was approved by the institutional ethical committee and carried out in accordance with the current guidelines for the care of laboratory animals. EPO or MSCs transplantation accelerated regeneration in SFI after one month and in all other parameters after two months. We suggest that EPO could act in a synergistic way with MSCs to potentiate their neuroprotective effect following peripheral nerve injury.

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). Erythropoietin is a hematopoietic cytokine, which has been shown to be expressed in the nervous system. It has also been shown that EPO possesses neuroprotective action in animal models of global and focal cerebral ischemia and spinal ischemia models in adult rodents (3,4). The effect of transplantation of mesenchymal stem cells in peripheral nerve injury combined with Erythropoietin has not been studied before. The aim of this study was to evaluate this combined effect on the improvement of the injured sciatic nerve in rats.

Materials & Methods

Study Groups				
G 1 (Control normal)	G 2 (Control injured)	G 3 (injured EPO treated)	G4(injured Mesenchymal SCs treated)	G5(injured combined treatment)
Intact Sciatic nerve (sham operated)	injured Sciatic nerve	erythropoietin (5000U/Kg) injected intraperitoneal once an hour after injury (5)	MSCs (2.5×10^4 cells/µl) grafted intralesional immediately after injury using insulin syringe	Same doses 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.

After eight weeks from the sciatic nerve injury

Walking track analysis was performed to all groups.

EMG using the Biopack, MP 150 system were done at 8th week post injury(9).

Rats were sacrificed and in vitro nerve conduction velocity was performed immediately .

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

Results

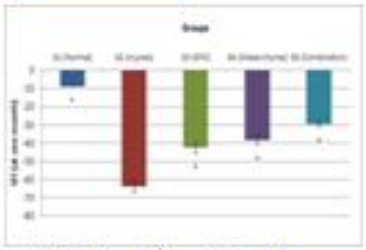

Figure 1: SFI values at 4th week in the study groups.

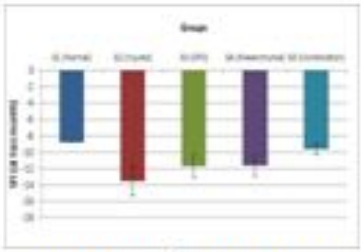

Figure 2. SFI values at 8th week in the study groups.

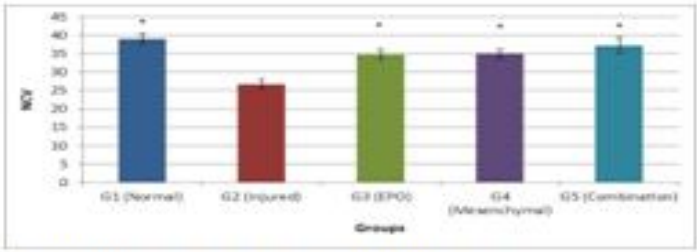

Fig.3 : Nerve conduction velocity values in mm/s in the study groups

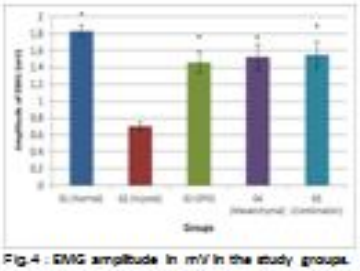

Fig.4 : EMG amplitude in mV in the study groups.

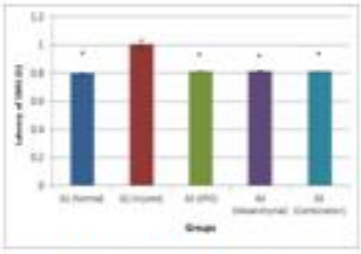

Fig.5 : EMG latency in S in the study groups.

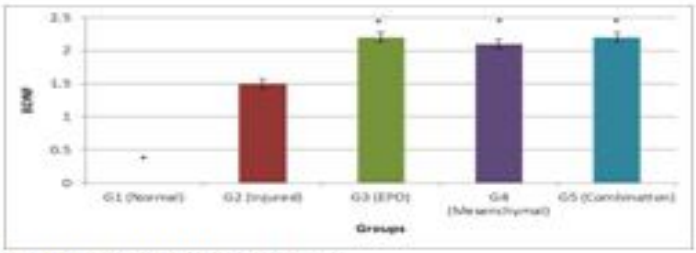

Fig. 6. BDNF mRNA levels in the study groups.

Conclusion

Treatment with Mesenchymal stem cells or with Erythropoietin can improve the behavioral, electrical and functional deteriorations occurring due to sciatic nerve injury in rat model. Combination of both regimens gives better improvement than using each one of them separately.

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