Indian Journal of Science and Technology, *Vol 9(40), DOI: 10.17485/ijst/2016/v9i40/103284, October 2016*
ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Morphological Characteristics by Cell Implantation After Spinal Cord Injury in Infarction Region

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Abstract

Objectives: Our research implanted stem cells to reduce behavioral deficiency in rodent animal models of clip compressive surgery inducing spinal cord infarction. **Methods/Statistical Analysis:** Non-transplanted control injection rats (n=10) were applied to spinal cord injury and administration of PBS (15 µl) after post-damage. Animals were injected with mESC implantation at the 5th day after injured surgery (n=20). **Findings:** We use the effect of grafted cell to the injured spinal cord region, focusing the use of mouse embryonic cells for regeneration of spinal cord infarction. These morphological characteristics postulated that mESC-graft could reduce the type of cavitations after damage in the SCI model. We also found that Schwann cell and glia cell contain numerous myelinated axons in the middle of the graft. **Improvement/Applications:** Our research suggests manifest result to prove that graft of stem cell could show behavioral improvement after spinal cord infarction.

Keywords: Clip-Compression, Embryonic Stem Cell, Graft, Infarction, Spinal Cord

1. Introduction

It has been reported that damaged nerve applied as grafted cell insertion into an infarction area, inducing from a clip compression damage of the spinal cord, enhances motor and behavioral function in injured rats, as tested by Locomotors testing scale method[1]. Various trial of cell transplant has been showed to enhance after spinal cord damage. We induce lesser the progress of additional damage, minimizing the injured-inhibitory condition of the infarction region, changing damaged tissue with transplanted cells, rebuilding Schwann cells and axonal regeneration, and stimulating specific growth factor and intrinsic progenitor cells[2]. Previous researches in rodents of spinal cord damage have been seen that stem cell transplants survived well in the infracted spinal cord, filled the cavitations region and found several neuronal cell types. Other studies showed that cell transplant improves motor functional recovery and induces the plasticity of related motor neurons[3]. It has been reported that spinal cord injuries, many molecular factors such as inflammatory cytokines, immune related proteins, and growth factors may contribute strongly to influence stem cell regeneration[4]. We postulate effective therapeutic strategy that stem cell transplant and positive neurological environment contribute to stem cell survival possibility to producing enhancement after spinal cord infarction.

2. Materials and Methods

2.1 Spinal Cord Infarction

Male SD rats were tested for this research (180–200 g, n = 30). This experiment was approved by the animal committee with policies of Namseoul University. We safely anesthetized with an i.p. insertion of pentobarbital sodium solution (30 mg/kg of total body weight). The animal surgery was treated under sterile conditions with safe experimental environment. The clip compression damage was applied to the site of the 9th to 10th thoracic spinal cord by exposing lamina. The vascular clip was been applied to spinal region in animal models[5]. Swelling urinary bladder was emptied by abdominal region pressure at three times

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daily. The animals were separated into a non-transplant group and a cell-transplant group. The transplant group was administrated with Mouse Embryonic Stem Cell (mESC) at 5-day post injury.

2.2 Implantation Methods

The non-transplanted group (n = 10) was tested to evaluate if solution amount (15µl) or implantation processing triggered specific locomotors differences in spinal cord injury rats. Non-transplanted control group was applied to spinal cord injury and administration of PBS (15µl). There was no motor damage in injured rats caused by the insertion processing. Animals were injected with mESC implantation at the 5th day after injured surgery (n = 20). Transplantation group was fully tested in animals (n = 20) with BBB scores below 2 at the 5th day after injury. The rest animals with BBB scores below 2, non-implant group (n = 10), had shown spontaneous recovery in behavioral assessment for 5 weeks period. A micro-injection was applied to graft intra-cellular quantification (1 x 10^6 cells) of cell suspension (15µl) using a 30-gauge needle on a 25 µl syringe linked on a micro manipulator. The 15µl volume of cell suspension was injected into the injury site or near the damage lesion.

2.3 Behavioral Assessment

Behavioral assessment was evaluated using the locomotors testing scale. Behavioral testing was maintained by a 20-min acclimation period daily to the experimental animals for the 35-day period of the testing.

2.4 Morphological Difference

All group rats have given intra-transcardially perfusion. Serial longitudinal sections (10 µm thick) were separated and each spinal cord section was treated with hematoxylin and eosin staining. Sections were also used for toluidine blue-stained, 10-µm thick plastic sections. The entire region of infarction was examined virtually and stained to distinguish the correct region of the infarction. The each section in the rostral and caudal directions of the injury region was prepared under at 40 x, 100 x and 200 x image through electron microscopy with H-E staining for a visual difference of cavitations.

2.5 Statistical Analyses

All values were scored as mean ±SEM. student's test was used to evaluate the overall difference between two groups at each point following injury. The level of significance for statistical analysis was setto less than 0.05.

3. Results

3.1 Behavioral Characteristics

The results of the BBB testing score in the spinal cord damage animals were shown in Figure 1 for the 35-day duration of the experiment. The BBB locomotors testing scores were measured daily in the non-transplant (n = 10) and transplant animals (n = 20). Transplantation group was tested in animals (n = 20) with BBB testing scores below 2 point at the 5th day after surgical damage. The rest of non-transplant group (n = 10) had shown spontaneous recovery in behavioral assessment in Figure 1. After the graft of cells, cell-implanted animals showed a significant functional enhancement of locomotors testing scores as compared to non-implanted control group animals at all times point examined.

3.2 Cavitation Characteristics

Control group rats with locomotors testing score less than 8 point showed the type of large cavitations morphology in Figure 2. The infarction size of cell-grafted group animals had cavities much smaller than the cavitations of control group animals in Figure 2. The morphological characteristics postulated that cell-grafting could reduce the size of cavities after damage in the infarction rodent models

3.3 Transplanted Cell Survival

The infarction site was showed regenerating cell survival with region of gray matter and white matter. The figures

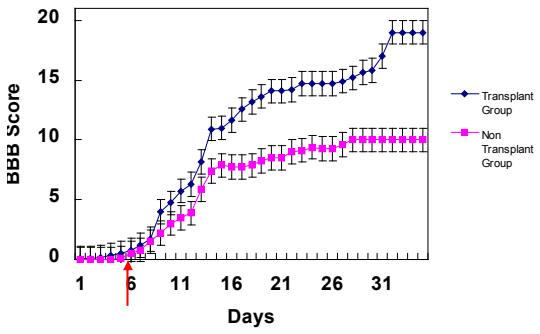
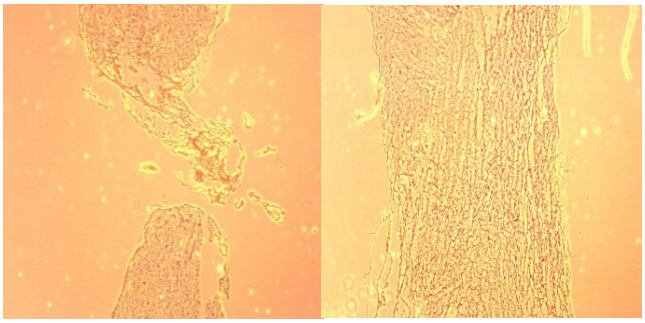


Figure 1: Differences in BBB test scores after surgical injury at spinal cord between non-transplant group and cell-transplant group. Cells were transplanted at the 5th day (indicated by arrow) after spinal cord injury.

contained many regeneration axonal neurons as well as various glia cells and Schwann cells in Figure 3. Electron microscopy showed the presence of various cell types.

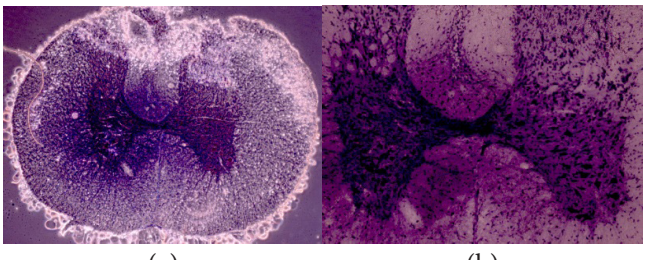
4. Discussion

Our study has demonstrated that the result of intra-cellular implant in the infarcted site is proved by effective and significant enhancement in the locomotor rating scores. Functional locomotive movement of the implanted group animals could be caused by a greater survival of regeneration axons at the injured region. Transplanted cell survival accompanied by small phenomenon of cavitation region may facilitate functional recovery[6]. Cell-implantation induced for at least five weeks post-transplant, partially filling the cavities and connecting into the intrinsic spinal cord and resulted in reduction of a large amount cavity formation[7]. It has been reported that reduction of cavity formation after spinal cord injury has been also experimented by graft of bone marrow stromal cells[8] and has been tested neural progenitor cells. The functional effects appeared in the mESC-grafted rats could not be due to the neuronal differentiation of grafted cells in the infarcted specific area, but due to the induction of various trophic factors beneficial to the regeneration processing including neuronal cells, glia cells and Schwann cells. A reduction of a damaged volume in the cavities of the injured site might be caused to the regeneration and reproduction of glial cells and Schwann cells as well as new neuronal cell formation at the damaged region of the infarction site[9]. Several kinds of trophic factor could contribute to influence the cavitation type of infarction size[10].



(a) (b)

Figure 2: Non-implant animals with BBB test scores less than 8 showed the formation of large cavities and disconnection. The spinal cord of transplant animals had cavities much smaller than the cavity situation of non-implant animals. (**a**): non-transplant animal, (**b**): transplant animal).



(a) (b)

Figure 3: Schwann cell and glia cell contain numerous myelinated axons in the middle of the graft. All sections show toluidine blue-stained, 10-µm thick plastic sections. (A: 40X magnification B:100X magnification).

5. Conclusion

Further research should specifically find that the synchronized interactions of various factors could be connected to the mechanism of the infarction processing. The result in this study showed that mESC-transplant contributed the functional and morphological recovery in the infarction rodent model.

6. Acknowledgement

This research paper is provided by Namseoul University financial fund.

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