Histological Difference by Cell Implantation of Spinal Cord Region from Infarction Surgery

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Abstract

Our experiment grafted stem cells to reduce behavioral deficiency in rodent animal models of clip compressive surgery inducing spinal cord infarction. Non-transplanted control injection rats were applied to spinal cord injury and administration of PBS after post-damage. Animals were injected with mESC implantation at the 5th day after injured surgery. Our research proved the effect of embryonic stem cells to the spinal cord infarction, focusing the application of grafted stem cells for regeneration of spinal cord nervous injury. These morphological characteristics postulated that mESC-graft could reduce the type of cavitation after damage in the SCI model. Our research suggests manifest results that implantation of embryonic stem cell could show behavioral improvement after severe spinal cord damage.

Keywords: Clip-compression surgery; Spinal cord injury; Implantation; Embryonic stem cell

Introduction

Previous studies had 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 & behavioural function in injured rats. It has been reported that administration of various stem cells applied into an injury-tissue area, resulting from a clip compression technique of the thoracic spinal cord region. These researches had suggested promotion of motor & behavioural function in injured animal models, as tested by the Basso, Beattie, Bresnan bar motor rating scale method [1]. Various trial of cell transplant has been showed to enhance after spinal cord damage and previous studies in rodent models occurring spinal cord damage have revealed that stem cell transplants survived in the damaged spinal cord region, filled the cavitation region and found several neuronal cell types [2]. Previous studies in animal models of Spinal cord injury have been shown that stem cell grafts grew well in the host spinal cord, filled the cavity and fused with the host spinal cord [3]. It has been observed that cell transplant improves locomotor recovery and modulates the excitability of motor neurons [4]. Following Central nervous system injuries, many molecules such as inflammatory cytokines, dead cell-derived proteins, and growth factors may interact to either positively or negatively regulate regeneration [5]. 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 [6]. 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. A therapeutic strategy that combines stem cell transplant and favorable neurological environment appears to be a particularly promising approach to enhancing recovery after Spinal cord injury.

Materials and Methods

Spinal cord injury experiment

Male SD rats were tested for this research (180-200 g, n=30). This experiment was approved by the animal committee with policies of Name Seoul 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. Swelling urinary bladder was emptied by abdominal region pressure at three times 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.

Implantation methods

The non-transplanted group (n=10) was tested to evaluate if solution amount (15 μl) or implantation processing triggered specific locomotor differences in spinal cord injury rats. Non-transplanted control group was applied to spinal cord injury and administration of PBS (15 μl). 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 10⁶ cells) of cell suspension (15 μl) using a 30-gauge needle on a 25 μl syringe linked on a micromanipulator. The 15 μl volume of cell suspension was injected into the injury site or near the damage lesion.
**Histological change**

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 & 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 40x, 100x and 200x image through electron microscopy with H-E staining for a visual difference of cavitation.

**Statistical analyses**

All values were scored as mean ± SEM. Student's test was used to determine the overall difference between groups at each point following SCI. The criteria for statistical significance was taken as p<0.05.

**Results**

**Behavioural characteristics**

The results of the BBB tests in the SCI animals were shown in Figure 1 for the 35-day period of the experiment. The BBB locomotion scores were assayed daily in the non-transplant (n=10) and transplant animals (n=20). Cell transplantation was performed in animals (n=20) with BBB scores below 2 at the 5th day after injury. The rest of non-transplant group (n=10) had shown spontaneous recovery in behavioural assessment.

![Figure 1: Changes in BBB scores after spinal cord injury between non-transplant group and transplant group. Cells were transplanted at the 5th day (indicated by arrow) after spinal cord injury.](image)

**Cavity change**

Control group rats with locomotor testing score less than 8 point showed the type of large cavitation morphology in Figure 2. The infarction size of cell-grafted group animals had cavities much smaller than the cavitation 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.

![Figure 2: Non-implant animals with BBB test scores less than 8 showed the formation of large cavities & 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).](image)

**Discussion**

The present study has demonstrated that the effect of cell transplant in the injured rat spinal cord is evidenced by significant improvement in the BBB scores. Alternatively, local axonal regeneration accompanied by significant reduction of necrotic cavitation may facilitate functional recovery [3,7]. 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 neural progenitor cells (NPCs)/neural stem cells (NSCs) [9,6]. 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. 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 & Schwann cells as well as new neuronal cell formation at the damaged region of the infarction site. The functional benefits observed in the transplant rats may not be due to the differentiation of stem cell in the injured tissue, but due to the production of trophic factors beneficial to the nervous tissue including neurons and astrocytes [10,11]. Although it is not determined at present what kinds of trophic factors are responsible for these phenomena, this influence might probably be due not to one specific factor, but to the synchronized actions of many factors. The data in this study demonstrated that stem cell transplant improved the functional and morphological recovery in the rodent model and we will use stem cell method of clinical application for the central nervous system injury patients in the future.
Conclusion

Our research has demonstrated that the result of intra-cellular implantation in the damaged animal spinal cord site is proved to an effective & significant enhancement in the BBB scale scores. Functional locomotive movement of the implanted group animals could be proved by a greater survival of regenerative axonal formation in the injured site. Future studies will strongly suggest that implanted cell survival resulted by significant reduction of neuronal cavitation tissue may facilitate motor and functional recovery of various central nervous system diseases as well spinal cord damage.

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