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Enhanced regeneration in spinal cord injury by the concomitant treatment of G-CSF and neuronal stem cells

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Running title: Augmented regeneration of injured spinal cord by neuronal stem cells and G-CSF

Key words: Neuronal stem cells, G-CSF, Spinal cord injury

Abstract

Purpose: G-CSF merits the properties of inhibition of programmed cell death and stimulation of neuronal progenitor differentiation. In addition, transplanted neuronal stem cells in injured spinal cord could survive; differentiate into astroglia and oligodendroglia, and support axon growth and myelination. Herein, we evaluate concomitant effect of G-CSF with neuronal stem cell on the spinal cord injury.

Material and Methods: Forty Sprague Dawley (n=10 in each group) rats weighing from 250-300gm were used in this study. Two millimeter transverse cord resection was carried over level of T8-9. Neuronal stem cell embedded in the fibrin glue either treated without or with G-CSF (50ug/KG x7 days) (group 3 and 4) and bare fibrin glue either without or with G-CSF (50ug/Kg x 7days) (group 1 and 2) delivered to gap of injured spinal cord were used for evaluation. Nerve regeneration was assessed by BBB scores, electrophysiology, histology and immunocytochemistry 3 months after the injury.

Results: Neuronal stem cells transplantation concomitant with the treatment of G-CSF (group 4) yield better regeneration than those treated either with neuronal stem cells transplantation (group3) or only G-CSF treatment (group2) according to the result of BBBS scores, motor evoking potential, and conduction latency. Better cord regeneration across the gap was only observed in those treated with neuronal stem cells and G-CSF (group 4). Higher densities of BrdU and better expression of Neu-N and MAP 2were observed in group 4 than those in group 2 or 3, but was no significant difference in expression of GFAP.

Conclusion: Concomitant treatment of neuronal stem cells and G-CSF could augment the regeneration in spinal cord injury better than that treated either with G-CSF or neuronal stem cells alone. The possible explanation of this synergic effect may be due to increased proliferation of progenitor cells and longer survival of neuronal stem

cells extrinsically or intrinsically.

Introduction:

The exact numbers of spinal cord injury (SCI) patients were not well known in Taiwan, but the estimated annual incidence of SCI was around 8.8 per one million people (1). The demand for long-term care services for SCI patients has thus become a major concern (2). Quadriplegia or paraplegia resulting from SCI causes devastating damage to the patient's life quality, his or her family, and the society. Microscopically, SCI leads to axonal degeneration, demyelination, activation of microglias and astrocytes, and death of neurons and oligodendrocytes (3). The general treatment strategies for SCI include surgical, pharmacological and physical therapies (4). The surgical intervention was aimed to renormalize the cord anatomy and repair the severed cord or nerve root by the procedure of decompression followed by instrumental fixation (5). Administration of methylprednisolone was recommended as a pharmacological therapy on the basis of the National Acute Spinal Cord Injury Studies, but it had little benefit on the recovery of the neurological function on injured patients (6). Physical therapy contributes to re-build up the neurological function, especially the muscular status (7). Up to date, severe SCI arising from cord ischemia and permanent cell death was still existed and this caused patient in the status of permanent neurological deficits, such as ventilator dependence, quadriplegia, paraplegia, and myeloradiculopathy. Even though modern medicine was enormously under development, we still can't conquer this obstacle to solve the problem existed for hundreds of years.

Current strategies employed by investigators to enhance regeneration in the injured spinal cord include neutralization of potential growth inhibitory molecules, transplantation of cells or tissue that support axonal elongation, and delivery of neurotrophic factors to promote axonal growth (8-10). Although transplantation of

fetal tissue has resulted in functional recovery in Parkinson's disease and Huntington's disease, clinical use of fetal cells is limited by the availability of donor tissue as well as logistic, immunological and ethical consideration (11). Neuronal stem cells (NSCs) as a source of multipotent graft tissue may be an alternative to fetal derived cells. NSCs can be isolated from embryonic or adult brain tissue, as well as differentiated from embryonic stem (ES) cells. NSCs are able to proliferate in vitro through many passages without losing their multipotentiality and can be induced to differentiate into enriched populations of glial or neuronal progenitors (12). There were enormous in vivo studies indicating that NSCs can differentiate into neurons and glial cells after being transplanted into the impaired brains or spinal cords (13-16). The fates of the engrafted cells predominantly differentiate into glial cells and fail to replenish the lost neurons caused by ischemia brain or SCI (12). Granulocyte Colony-Stimulating Factor (G-CSF), a key hemopoietic factor of the myeloid lineage, has been extensively used for more than 10 years in the treatment of neutropenia as well as for bone marrow reconstitution and stem cell mobilization (17). Apart from these well-known activities, a series of reports have demonstrated that G-CSF displays immunoregulatory properties: G-CSF is able to expand the monocyte/macrophage subset and to promote an anti-inflammatory pattern conferring protection in murine endotoxemia (18). A similar shift in cytokine production pattern has been recently confirmed in human peripheral blood T cells (19). Yoon and associate reported that G-CSF had the neuronal protection against apoptosis in spinal cord injury and exerted its effects through G-CSF receptor on the neuronal cell (20). Appropriate intervention on the spinal cord injury should depend on the nature, extent, and duration of a particular disease state, as pathophysiology can dramatically evolve over time. Immediately after spinal cord injury, the initial mechanic damage if followed by a cascade of potentially harmful secondary events that include the

formation of free radicals, detrimental inflammatory response and death of neurons and glia. Attenuation of proimflammatory cystokines such as TNF-α, interleukin 1 β after could promote neurogenesis and lessen gliosis (21). At later time point, interventions must address those processes to target functional regeneration of severed axons and restoration of interrupted axons. Iwanami and associates reported that the grafted human neural stem cells survived and differentiated into neurons, astrocytes, and oligodendrocytes to restore connection of spinal cord (22). Thus, the concomitant treatment with anti-apoptotic and anti-inflammatory agent such as G-CSF and supplement of neuronal stem cells no only has the effect on suppression of detrimental effect by injury but also supply progenitor cells in restoring cell loss. In this study, we repair the transverse spinal cord injury with G-CSF concomitant with neuronal stem cell to evaluate the possibility of this strategy in spinal cord injury.

Material and methods:

Preparation of Neural Stem Cells (NSCs)

The rat NSCs will be prepared according to Chiang et al. (22). Pregnant Sprague-Dawley rats with 14- or 15-day-old fetuses are deeply anesthetized by ether. The fetuses are removed from the uterus and put into cold Hank's Balanced Salt Solution (HBSS) (GIBCO, NY). The brain areas of cerebral cortex, striatum and mesencephalon are dissected carefully. These tissues are cut into small pieces and then washed with HBSS twice before being put into 1-2 ml of protease-papain-DNase solution (PPD), which consists of 10 mg papain, 100 mg protease, 10 mg DNase 1, and 12.4 μl 1M $MgSO_4$ in 100 ml HBSS. After 30 min incubation at 37℃, gentle trituration with a Pasteur pipet is performed 10 times. The entire procedure is repeated once and then the PPD is replaced by Dulbecco's modified Eagle's medium (DMEM)/F12 nutrient (1:1, GIBCO, NY) containing DNase 1 (1%, w/v). After 15

minutes incubation, gentle trituration is performed another 10 times. The dissociated single cells are collected by centrifugation at 1500 *g* for 5 min. The cells are resuspended in DMEM/F12 medium containing N2 supplement (25 mg/ml insulin, 100 mg/ml transferrin 20 nM progesterone, 60 mM putrescine, and 30 nM selenium chloride; Sigma) and cell counting is performed with a hemacytometer. Viability is checked by trypan blue exclusion method. A plating density of 50,000 cells/cm^2 is used to culture epidermal growth factor (EGF)-responsive neurospheres in non-coated 75 cm^2 flasks with N2 defined medium containing 20 ng/ml EGF (GIBCO, NY). EGF is added every 4 days and the medium is changed every 2 weeks. No passage is performed on these neurospheres.

In vitro Bromodeoxyuridine labeling

BrdU (50 μg/ml; Sigma) was added to each shell vial to a final concentration of 10μM and incubated with NSCs at 37°C for 2 hours before transplantation.

Spinal Cord Injury and Transplantation of NSCs or G-CSF Treatment

Adult female Sprague-Dawley rats (250-300 g) are anesthetized with 4% halothane and placed in a prone position. Anesthesia is maintained with 2% halothane. The body temperature is monitored and kept at 37°C by a temperature controller. Using aseptic surgical techniques, an incision extending from the mid to low thoracic regions on the dorsal side is made. Laminectomy of the caudal portion of the T8 and T9 spine is performed under the microscope to expose the spinal cord. Spinal cord injury is then produced by transection of the cord at the level of T8-T9.

For animals subjected to transection, the spinal cord is completely severed with microscissors between the two stumps of the cord. The vertebrae (T7-10) around the lesion are fixed with Ti-cron to the proximal ribs (T7-10), and transverse fixation of the T7 and T10 vertebrae to the corresponding ribs was also made. 10 μl of NSCs (50,000 cells/μl) are injected into the space between the T8-T9 (n=10 per group). The lesion cavity is covered serially with fibrin glue and a piece of gelfoam. Fixation of

the vertebrae and ribs was also made as described above. One day after produced of SCI, G-CSF treatment groups and NSCs with G-CSF groups (n=10 per group) were injected subcutaneously with recombinant human G-CSF (50μm/kg per day; Amgen Biologicals) once daily for 5 days.

The surgical site is sutured layer-by-layer and the animal is then allowed to awake. Postoperative care includes manual bladder compression 2-3 times per day, injection of lactated Ringer's saline for dehydration, food supplement for weight loss, administration of antibiotics for bacterial injection, and visual inspection for skin irritation or decubitus ulcers.

In vivo Bromodeoxyuridine labeling

Bromodeoxyuridine (BrdU), a thymidine analogue that is incorporated into the DNA of dividing cells during S-phase, was used for mitotic labeling (Sigma Chemical). Pulse labeling was used to observe the time course of proliferative cells in the spinal cord injury. Five days before sacrificing, each animal received intraperitoneal injection of BrdU (50 mg/kg) for the five consecutive days.

Neurophysiological Study

The motor evoked potential (MEP) and conduction latency will be studied before the animal sacrificed 3 months after the injury. In brief, the rats underwent anesthetizing and one stimulating electrode was fixed on the dural surface of the motor area (3 mm lateral and 2 mm posterior to the bregma). Electric stimulation mA) was applied to the stimulating electrode and the reference electrode was placed on the opposite side skull; the conduction latency, and the compound muscle action potential were recorded with an active electrode needle 10mm below the tibia tubercle and a reference needle 20 mm from the active electrode. The stimulation intensity and filtration range were 20mA and20-2000 Hz, respectively.

Assessment of Hind limb Motor Function

Four different tests (n=10 per group) were used to evaluate the function after SCI by a technical assistant who was blinded to treatment allocation evaluated every week after the surgery. The hind limb motor functions will be assessed with BBB locomotors rating scale described by Basso, Beattie and Bresnahan (23). BBB scores will be recorded before operation and once weekly after operation until the sacrificing of animals. With this 21-point scale, animals achieving scores of 1-8 are capable of hind limb joint movements without weight support. Those in ratings of 9-13 demonstrate varying degrees of hind limb weight support and forelimb-hind limb coordination, and those achieving scores of 14-21 show improvement in paw and tail position, toe clearance, and trunk stability during a fully supported and coordinated gait.

Immunohistochemistry of the Spinal Cord

Three months after the injury, 10 rats in each group will receive transcardial perfusion with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). The spinal cords are removed and dissected by cutting 1-cm segments, around and rostral and caudal to the lesion center. These 1-cm tissue blocks are postfixed in 4% paraformaldehyde for 4 h and cryoprotected in 20% w/v sucrose in phosphate buffered saline (PBS) for 20 h. The tissues are embedded in Tissue-Tek OCT medium and cryosectioned at 10-μm thickness. After rinsed with PBS, the frozen sections are blocked with 5% skim milk in PBS for 30 min. Then they are incubated overnight at 4°C with primary antibodies. Primary antibodies used were directed against the following antigens (using the following dilutions): (1) microtubule-associated protein 2 (MAP2; 1:500; Neromarkers) for neurons; (2) glial fibrillary acidic protein (GFAP; 1:500; Becton, Dickinson and Company) for astrocytes; (3) NeuN (1:1500; Chemicon) for neurons;(4) BrdU (1:400; Boehringer Mannheim) for nuclear identification. Subsequently, the sections are rinsed with PBS and incubated with fluorescein isothiocyanate (FITC)- or rhodamine (TRICT)- conjugated secondary antibody for 1 h at room temperature. The immunostained sections are examined under a fluorescence

microscope.

Statistical analysis

All values will be expressed as mean ± SEM. Differences among groups will be first compared by one-way analysis of variance (ANOVA) and then analyzed by Duncan's test. $p<0.05$ is considered statistically significant.

Results:

Motor Function Evaluation

BBB scores in four treatment groups plotted against the time with interval of one week were shown in Figure 1. Before the 8- week follow- up, there was no statistical significance of BBS scores in group 2, 3, and 4. At 12- week follow- up, BBB scores in four groups were 3.86(SE 0.26), 7.89(SE 0.20), 9.625(SE 0.26), and 11.67(SE 0.17), respectively, which revealed the statistical significance ($p<0.001$). The post Hoc studies showed the significance in group 1 and 2($p<0.001$), group 2 and 3($p<0.001$), and group 3 and 4($p<0.001$).

Twelve 12 weeks after the injury, the motor evoke potential (MEP) in four respective groups were 24.69(SE 3.51), 31.64(SE 3.06), 38.97(SE 2.30), and 47.7(SE 3.17) mV illustrated in Figure 2. The discrepancy showed the statistical significance ($p<0.001$). The Post Hoc examination showed the statistical significance between group 2 and 3 ($p=0.04$), group 2 and 4 ($p=0.002$), group 3 and 4($p=0.02$).

At 12- week follow- up, the conduction latency in four respective groups were 1.54(SE 0.04), 1.39(SE 0.03), 1.39 (SE 0.04), and 1.29(SE 0.02) shown in Figure 3. The difference in conduction latency showed the statistical significance ($p<0.001$). The post Hoc test showed the significant difference in group 1 and 2 ($p=0.003$), group 2 and 4($p=0.005$) and group 3 and 4($p=0.002$), but there was no statistical significance between group 2 and 3 ($p=0.1$).

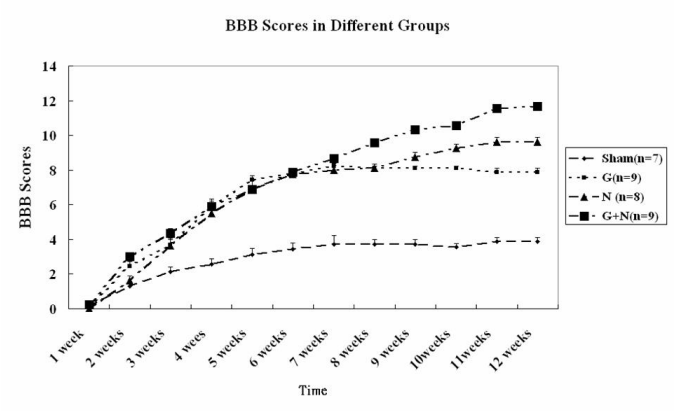


Figure 1: BBB scores were plotted against with time.

Before 8-week follow up, injured rats either treated with G-CSF (group 2), neuronal stem cell (group 3), or combined (group 4) benefit better than those without any treatment in BBB scores. There was no significant difference between these three groups. At 12 weeks follow-up, rats in group 4 had higher scores than those in group 3 or 4($p<0.001$) and groups 3 achieved higher scores than those in group 2($p<0.001$). Sham: filled the gap with fibrin glue; G: fibrin glue+ subcutaneous G-CSF; N: fibrin glue+ neuronal stem cells; G+N: fibrin glue+ neuronal stem cells+ subcutaneous G-CSF

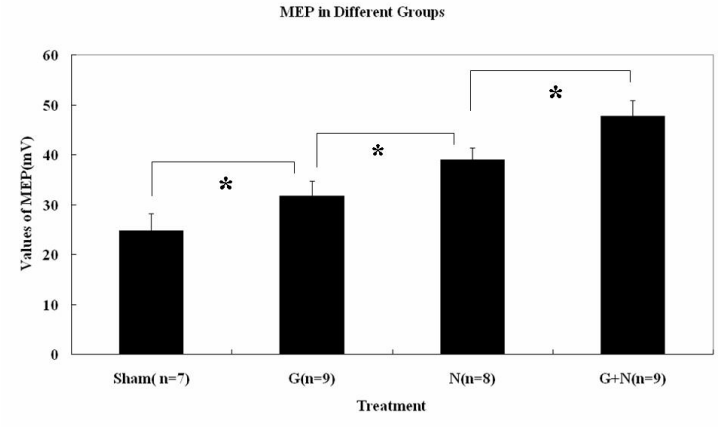


Figure 2: Value of motor evoked potential (MEP) was illustrated at 12- week follow

up. MEPs in group 4 had higher value than those in group 3(p=0.02) , group 3>group2(p=0.04), and group 2>group 1(0.05).

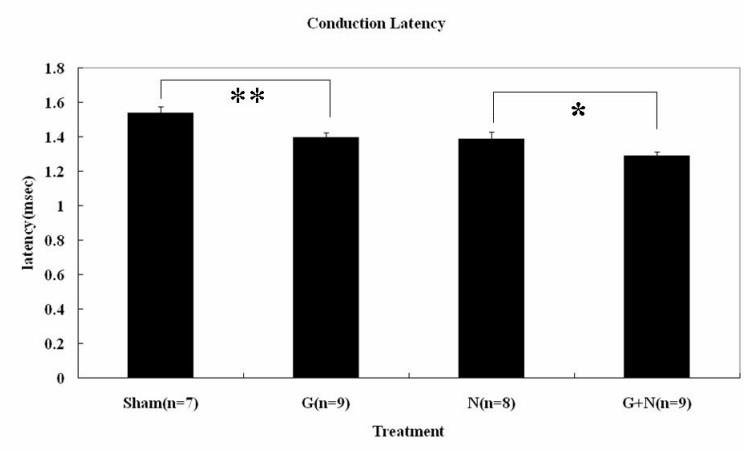


Figure 3: Conduction Latency was illustrated in different treatment modalities. Conduction latency in group 4 was shorter than those in group 3(p=0.02) as well as those in group 2 < group 1(p=0.003). There was significant difference between group 3 and 2.

Group 1, 2, 3, and 4: see the text

Histocytochemistry in injured area

In the histology examination in the injured area, there was well regeneration tissue bridging the distal and proximal end of spinal cord only observed in those treated with neuronal stem cells combined with G-CSF illustrated in Figure 4. The staining and count of BrdU in the injured cord was illustrated in Figure 5A and Figure 5B, respectively .The counts of BrdU staining in four groups were 0(SE 0), 18.4(0.51), 17.8(SE 0.37), and 26.8(SE 10.7), respectively, which revealed the statistical significance($p<0.001$). The post Hoc examination showed the statistical significance in group 1 and 2($p<0.001$), group 3 and 4($p<0.001$), but there was no statistical significance between group 2 and 3 (p=0.02).

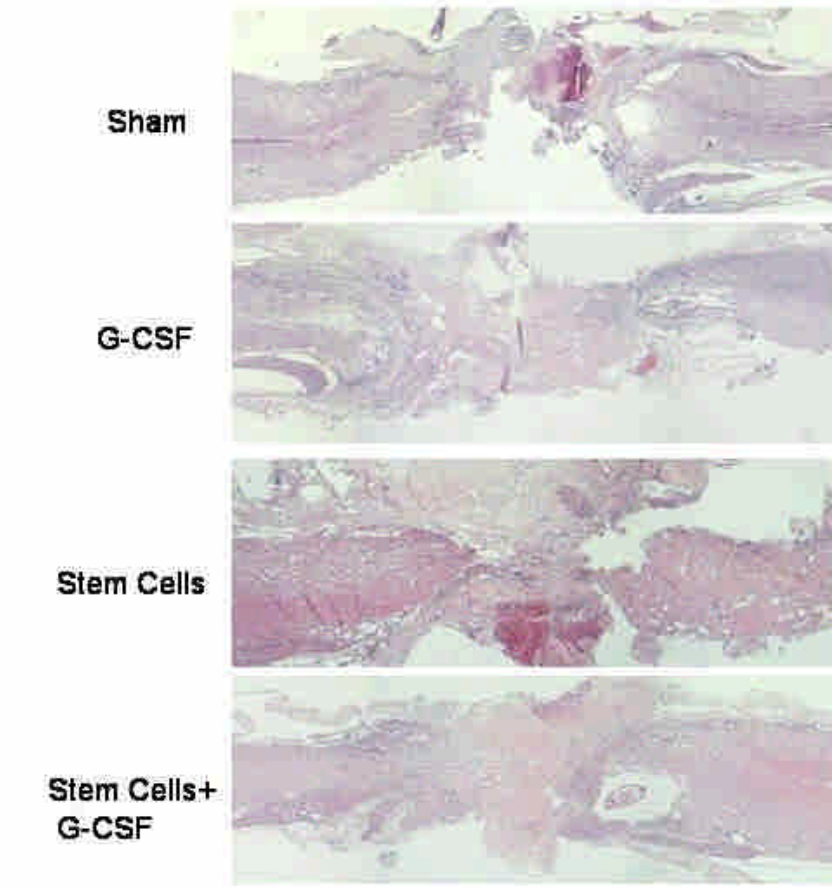
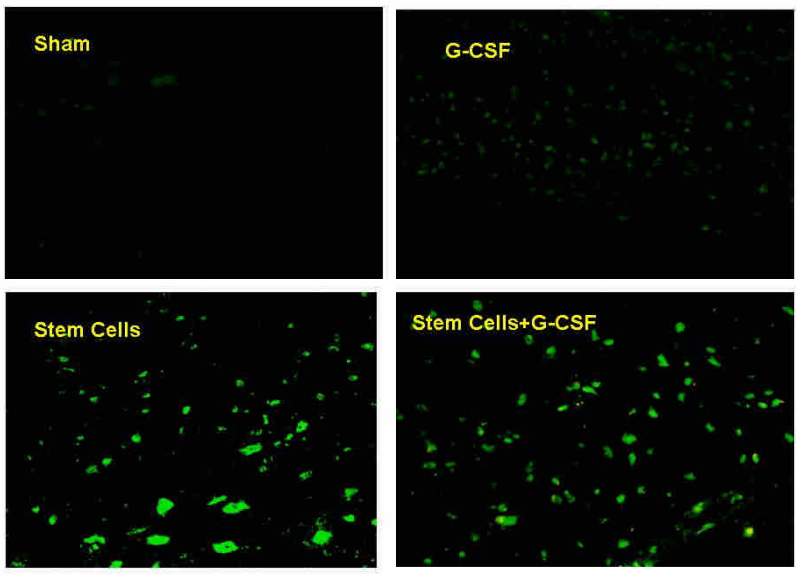


Figure 4: Illustration of pathological finding was depicted in four groups. Well organized tissue bridges the gap was only observed in group 4. There was no significant between group 1, 2, and 3.

A



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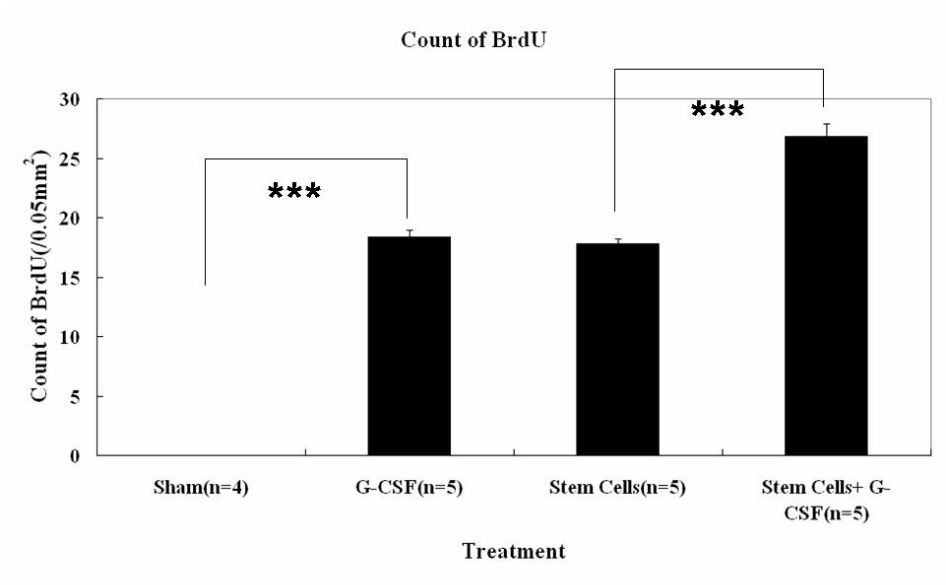
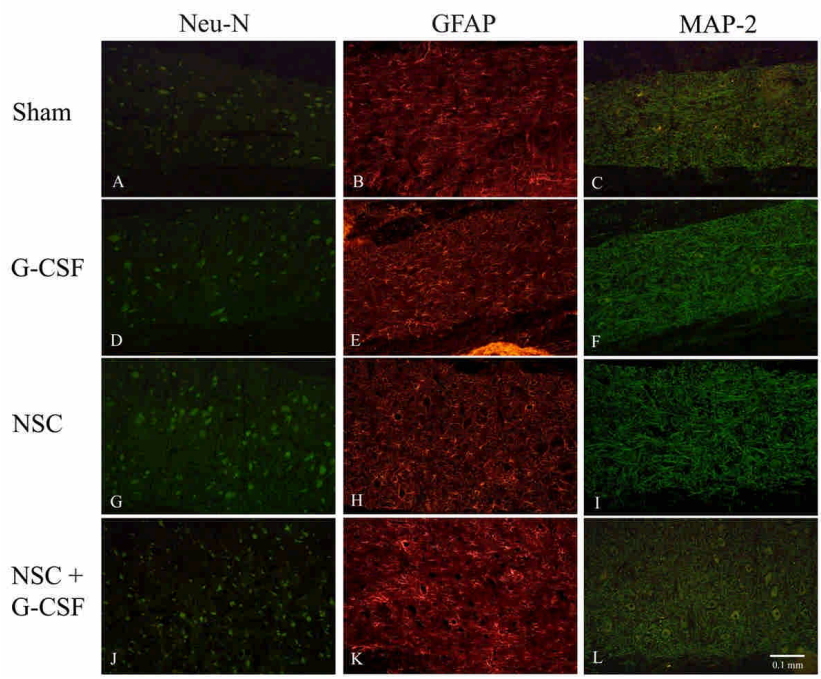


Figure 5: Staining and count of BrdU was illustrated in repaired area. (A) Group 4 showed higher density than those in group 3 and 2. The size of BrdU staining in group 2 was smaller that in group 2, but there was significant difference in cell density. (B) Counts of BrdU in group 4 were higher than those in group 3 ($p<0.001$) as well as those in group 1 < group 2($p<0.001$). There was significant discrepancy in group 3 and 2 ($p=0.2$).

Immunoctochemsitry in distal end of transverse spinal cord injury

The immunocytochemistry studies over the distal end of injured spinal cord including Neu-N, GFAP ,and MAP-2 were illustrated in the Figure 6A. Higher expression of Neu-N and MAP-2 was observed in group 4 than those in group 3 and 2 as well as those in group 2>1. The counts of Neu-N in four groups were 11.57(SE 0.48), 19.33(SE 0.33), 25.75(SE 0.99), and 34.56(SE 0.50) illustrated in Figure 6B. The discrepancy showed the statistical significance ($p<0.001$). The post Hoc examination revealed the statistical significance in group 1 and 2($p<0.001$), group 2 and 3($P<0.001$), and group3 and 4 ($p<0.001$).

A



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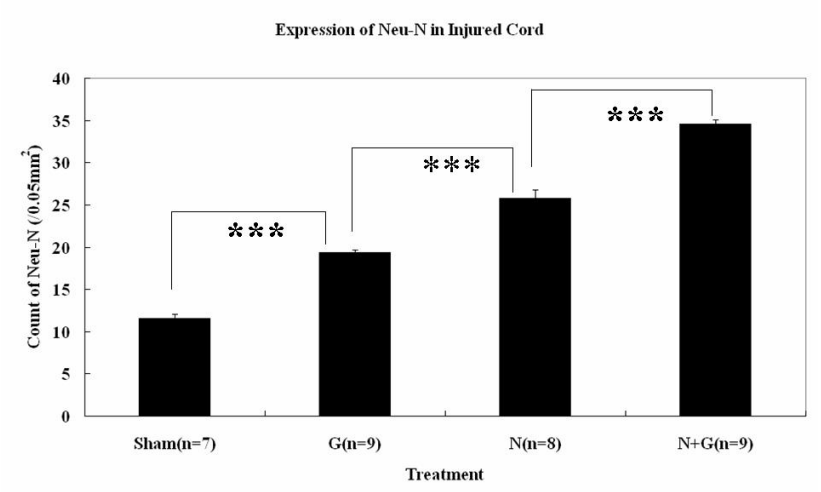

Figure 6: Expression and counting of Neu-N, GFAP and MAP-2 were demonstrated in different treatment group (A) Higher expression of Neu-N, MAP-2 was observed in group 4 than those in group 3 as well as group 3>2 and Group 2> group 1. Better expression of GFAP was shown in group 4, 3, 2 than those in group 1 (B) The

counting of Neu-N in five consecutive area demonstrated those in group 4>3(P<0.001), group 3>2(P<0.001), and group 2>1(<0.001).

Discussion

Strategy related to the pathophysiolgoy of SCI

Every year, almost 10000 new cases of spinal cord injury (SCI)-associated disability are reported for which only the only clinical approved treatment available at present is high dose methlyprednisolone. Post traumatic inflammatory reactions play an important role in secondary injury after SCI. After experimental SCI, transcripts of proinflammatory cytokines such as tumor necrosis factor α(TNF-α), and interleukin1 β(IL-1 β) are up regulated (24). TNF-α and IL-1 β are both potent inducers of inducible nitric oxide synthetase(iNOS), and their expression during SCI possible results in upregulation of iNOS in macrophage and astrocytes (25).The pathologically high level of NO produced by iNOS in the central nervous system are associated with inhibition of mitochondrial function, rapid glutamate release from both astrocytes and neurons, and excitotoxic death of neuron (26). Attenuation of post-inflammatory cytokines such as TNF-α ,IL-1 β, and iNOS provided protection against SCI-induced tissue necrosis, neuronal and olgiodendrocyte apoptosis, demyelination, and reactive gliosis and resulted in remarkable improved functional outcome after SCI (20, 21).

Ideally, cellular candidates for transplantation should be able to replace the function of astrocytes, which build the cellular scaffold of spinal cord parenchymal and can provide guidance cues of regeneration axons andoligodendrocytes, which myelinate axons, thus allowing proper nerve conduction. Various cell types such as fibroblast, Schwann cells and olfactory ensheathing cells have been analyzed for their regenerative capacity after transplantation into the injured cord (30-32). Cell based therapies are able to substitute for the loss of glia cells to some degree and to mediate the application of growth promoting factors; however, structural and functional

recovery was moderate at best.

There was no single intervention to fulfill the various status of SCI such as nature, extent and duration of particular disease state. Base on the strategy of delivery of anti-inflammatory agent and supplement of cell loss, we designed the treatment protocol by using concomitant of neuronal stem cell transplantation and anti-inflammatory and anti-apoptotic agent to treat the spinal cord transverse resection injury. Result from this study demonstrated that rat treated by the concomitant cell transplantation and G-CSF achieve better outcome in locomotor rating scales, motor evoked potential, and conduction latency. Furthermore, well bridged tissue and high proliferation cells located the injured gap was observed than those either with neuronal stem cell transplantation or G-CSF treatment. High expression of neuronal and glia cell maker was also detected adjacent to the injured cord. It seemed that the concomitant treatment of cell based-therapy and delivery of G-CSG may be an alternative therapy option in patient with SCI.

Effect of GCSF on SCI

G-CSF is a 19.6kDa glycoprotein common used to treat the neurtropenia (7). Known sources of G-CSF in the body include monocytes, methothelial cells, fibroblasts, and endothelial cells, and receptors for G-CSF are present on precursor and mature neutrophilic granulocytes, monocytes, platelets, and endothelial cells. At the myeloid progenitor cell level, G-CSF stimulates the growth of neutrophic granulocyte precursors (27). G-CSF crucially regulate survival of mature neutrophil(28) by inhibition of apoptosis (29). G-CSF displayed strong anti-apoptotic activity in mature neurons and activate multiple cell survival pathway. Both G-CSF ant its receptor are widely expressed by neuron in CNS, and their expression is induced by ischemia, which suggests an autocrine protective signaling mechanism. Surprisingly, the G-CSF receptor was also expressed by adult neuronal stem cells, and

G-CSF induced neuronal differentiation in vitro. G-CSF markedly improved long-term behavioral outcome after cortical ischemia, while stimulating neural progenitor response in vivo, providing a link to functional recovery (33). In the other hand, Park and associates reported that G-CSF generate sensimotor recovery in intracerebral hemorrhage by reducing brain edema, decreasing inflammation reaction, and avoiding perihematoma cell death(34). G-CSF receptor not only existed in the brain but also was demonstrated in spinal cord. Treatment of experimental spinal cord injury with G-CSF alleviated neuronal cell death in spinal cord due to its antiapopotic effect (20).

Collectively, the benefits of G-CSF administration included the proliferation of neural progenitor cells, anti-inflammatory effect and anti-apoptotic effect on neuronal cell survival. The present study revealed that G-CSF administration in spinal cord transverse resection injury could augment the improvement in locomotor function scale and electrophysiological parameter due to higher proliferation cells in the injured site as well as better expression of neuronal and glia marker over peri-injury area.

Cell supplement in SCI

The replacement of neurons is negligible in spinal cord injury, as only neurons at the injured segmental level are lost and therefore contribute only minimally to the functional deficits observed after spinal cord injury. Organotypic cell replacement can be achieved with neural progenitor cells (NPC). NPC from embryonics as well as adult central nervous system tissue have the capacity for self renewal and multipotency. After delayed transplantation of embryonic derived NPC into injured rat spinal cord differentiation into glia and neuronal lineage as well as modest functional improvement have been reported (15). However, ethical concerns and limited availability restrict the large-scale use of embryonic –derived NPC. To prevent

rejection of allogenic embryonic cells after transplantation, suppression of the host immune system is required, which represent another major disadvantage of embryonic derived neural cell grafts. In contrast, the patient's own cells could be utilized to obtain adult NPC, thus avoiding issues regarding chronic immunosuppression and ethical concerns, as would apply for embryonic cell sources. Transplantation of adult NPC into the intact spinal cord of adult rats revealed that grafted cells survived, migrated over considerable distance within spinal cord and differentiated into astroglial and oligodendroglial cells (35). Grafted into injured spinal cord, adult NPC will survive, differentiate into oligodendroglia and astroglia cell leanage, integrate them along axon pathway for poetential remyelination and support axon growth (36). In our study, we observed that NPCs grafting yielded higher density proliferation cells in the injured spinal cord as compared those without cell transplantation. This higher proliferation cells may be the transplanted cell or migrated macrophage. Higher expression of neuronal and glia cells markers adjacent to the injured site paralleling better functional outcome implicated that neuronal cell transplantations could support axon growth and remyelination.

Concomitant treatment in SCI

Theoretically, concomitant administration of anti-inflammaotry/ anti-apopotic agent with cell supplement obeys the nature, extent and duration of spinal cord injury. In the present study for rats undergoing the combined therapy, the functional outcome such as BBB scale, motor evoked potential, and conduction latency generated the favorable results than those either treated with G-CSF or neuronal stem cells alone. Higher cell proliferation and better expression of neuronal cell marker was also observed which it indicated this addition effect arising from the anti-inflammatory/anti-apoptotic effect of G-CSF on the transplanted cells.

Upcoming studies

The present study demonstrated that either neuronal stem cell transplantation or G-CSF administration or the combined therapy could enhance regeneration in spinal cord injury. But, how synergic effect worked was not answered in this study. Further studies included the investigation of G-CSCF receptor and anti-apoptotic staining in the transplanted cell and measurement of cytokines expression after administration of G-CSF.

Conclusion

Combined therapy with neuronal stem cells and G-CSF yields better results than those either with any single treatment. This alterative therapy provided anther milieu for patient with spinal cord injury. This concomitant effect was not fully investigated and should need further studies.

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