11/29/06

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Progress Report for NJCSCR (Yu-Wen Chang)

Overall Plan Summary:

Traumatic injury to the spinal cord initiates a cascade of degenerative processes, known as secondary injury, which include various inflammatory reactions. Anti-inflammatory drug Methylprednisolone (MP) has been a standard treatment to SCI patients and shown to preserve some neurological function if administered within 8 hours after the injury. In animal models, MP also has been shown to reduce lipid peroxidation, protect neurons, and promote functional recovery. Minocycline (MC), a tetracycline derivative, has been reported to have neuroprotective effects in animal models of neurodegenerative diseases, ischemia, and brain injury. The major effect of minocycline is to block cell death pathway and to reduce inflammation. In spinal cord injury models, minocycline reduces cell death and inflammation therefore improves functional recovery. Our previous study of acute transplantation of RG3.6 showed improved functional recovery at early time points (~2d). Given the beneficial effects of anti-inflammatory therapy, we hypothesize that a combination of radial glial transplantation with anti-inflammatory treatment may yield greater improvement than either one alone. Our rationale is that stabilization of traumatized tissue by anti-inflammatory therapy in the acute phase will prevent trauma induced cell death and facilitate implanted radial glia function in a way similar to developing brain to bridge the injury, guide damaged axons, promote axonal regeneration, and further contribute to behavioral recovery.

Progress Summary:

During the reporting period, we have conducted acute administration of anti-inflammatory drug MP or MC followed by 9-day delayed transplantation of radial glial clone RG3.6 cells to rat with contused spinal cord injury. The purpose of this period of work was to select a better anti-inflammatory drug for chronic study. Migration of transplanted cells was measured and showed no significant difference in rats treated with or without anti-inflammatory drugs (Task 1). We proposed that acute administration of MP or MC may reduce the number of activated macrophages, which may prevent secondary injury (Task 2). Locomotor recovery was determined by BBB score and only showed a slight increase in rats that received MP or MC at day 2 post-SCI (Task 3). Spared white matter was quantitated and MP or MC treated rats preserved more white matter at rostral regions to the epicenter (Task 4). Oligodendrocyte labeling by CNPase antibody showed more oligodendrocytes in MP treated rats, suggesting MP reduces delayed oligodendrocyte death (Task 5). The behavior results did not show significant improvement, however histological observations indicate this combination therapy can protect oligodendrocytes and prevent Wallerian degeneration.

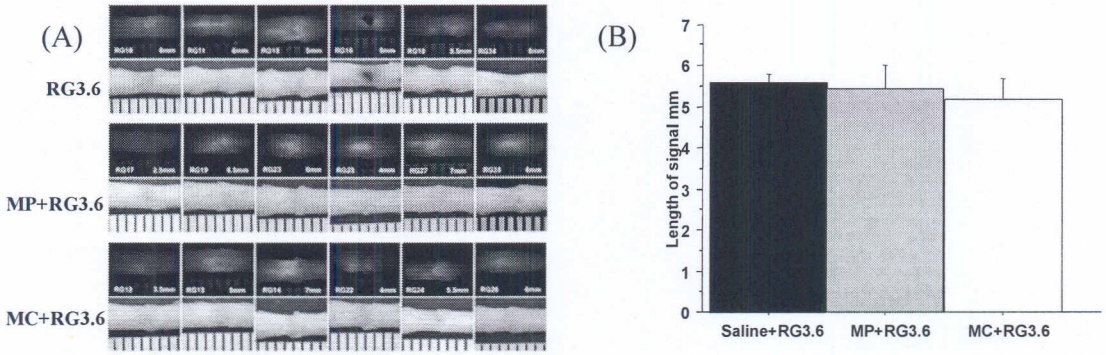
Although NJCSCR has terminated this fellowship for the reminder of grant period, the results of oligodendrocytes and myelin preservation motivated us to continue this project and focus on optimizing the therapeutic time window to combine MP with transplantation of RG3.6 cells. We modified our experiment design from 9 day delay radial glia transplantation to 0-2 day sub-acute transplantation with single injection of MP following contusive spinal cord injury.
In view of BBB scoring system can not represent as a whole recovery index, we also conducted footprint and grid walking testes to evaluate animals' walking improvement.
In task 6, we conducted acute MP administration with acute or sub-acute RG3.6 transplantation after spinal cord injury. Significant improvement was observed in rats received acute MP and sub-acute RG3.6 transplantation. From footprints and grid walking results, we also found that 0-48 hours post injury is an effective window for radial glial transplantation.

We did not continue any minocycline study because of severe liver fibrosis problem was found in some rats and may affect our evaluation on their recovery after spinal cord injury.

Specific tasks used in this project

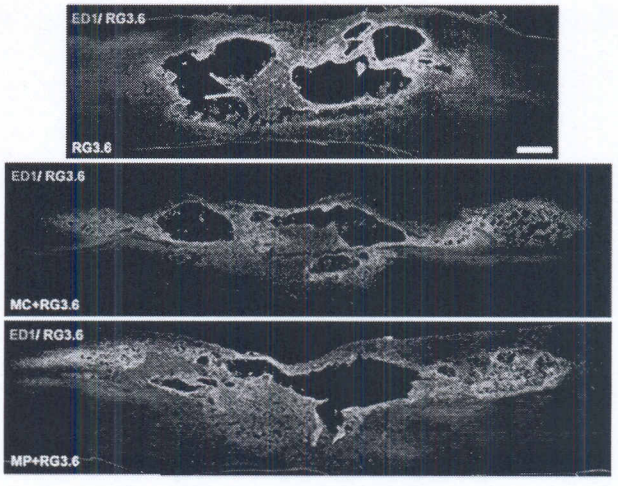
18 rats were randomly assigned as 6 control rats which were given saline or PBS through neck external jugular or intraperitoneal injection, 6 MP rats which were injected with MP through jugular vein within 5 minutes after contusion, and 6 MC rats which were given MC through intraperitoneal injection within 5 minutes after contusion. 9 days post injury RG3.6 cells were injected to the injury site, and rats were euthanized 7 days after cell transplantation.

(1) Transplanted cells migration



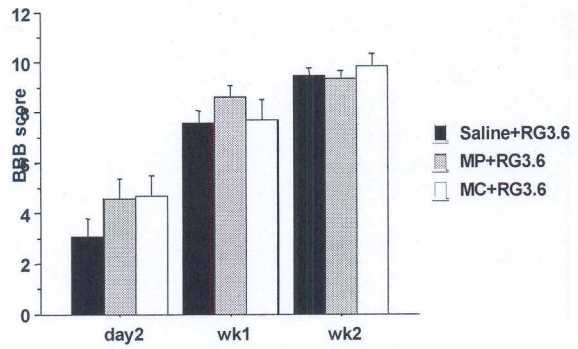
We proposed that immediate administration of MP or MC can stabilize tissue after contusive injury and facilitate survival and migration of subsequent RG3.6 cells transplantation. 7 days after transplantation of RG3.6, green fluorescent protein (GFP) signal was used to analyze transplant distribution and signal length from rostral to caudal was measured using fluorescent dissecting microscopy (A). RG3.6 cells appear to have similar migration extents in all conditions. (N=6 for each group, data present mean ± SEM)

(2) Microglia and activated macrophages



Microglia or activated macrophages were immunostained by ED1 antibody and showed no reduction in MC or MP treated rats 16 days post SCI (7 days after RG3.6 implantation). Scale bar = 500μm.

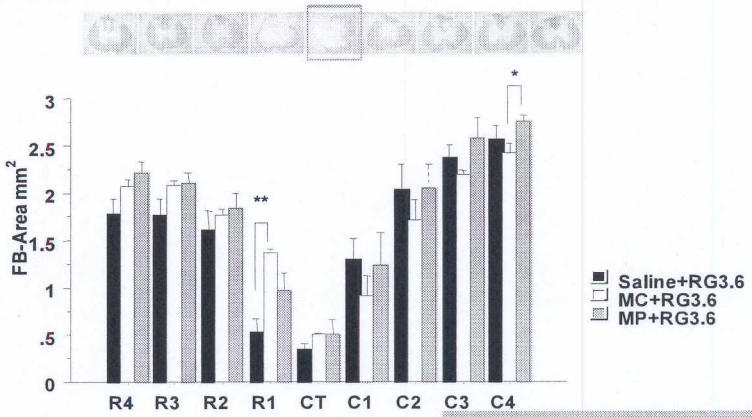
(3) Locomotor recovery measured by BBB score



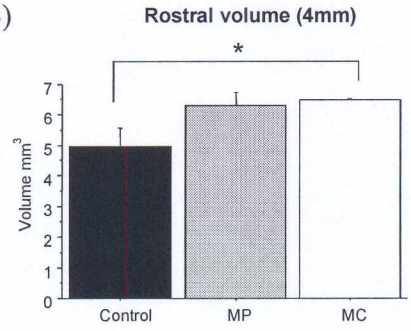
Locomotor recovery was measured using BBB score scale at day 2, week 1 (before cell transplantation) and week 2 (after cell transplantation). There is a trend that MP or MC rats have higher scores than controls at day 2, but overall the results are not statistically significant. Our previous study of acute transplantation of RG3.6 showed a score 8 to 9 at week 1 (plantar stepping to dorsal stepping) (Hasegawa, Chang et al. 2005), however, in this study, we did not find that MP or MC alone increase the score significantly at 1 week. The combined results suggest that acute transplantation of RG3.6 cells alone may be effective in protecting spinal cord from secondary damage and in promoting functional recovery. (N=6 for each group, data present mean ± SEM).

(4) White matter sparing

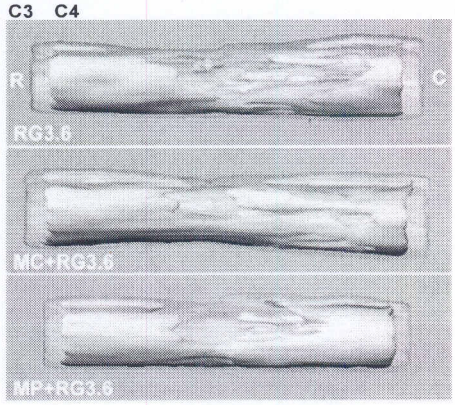
(A)



(B)

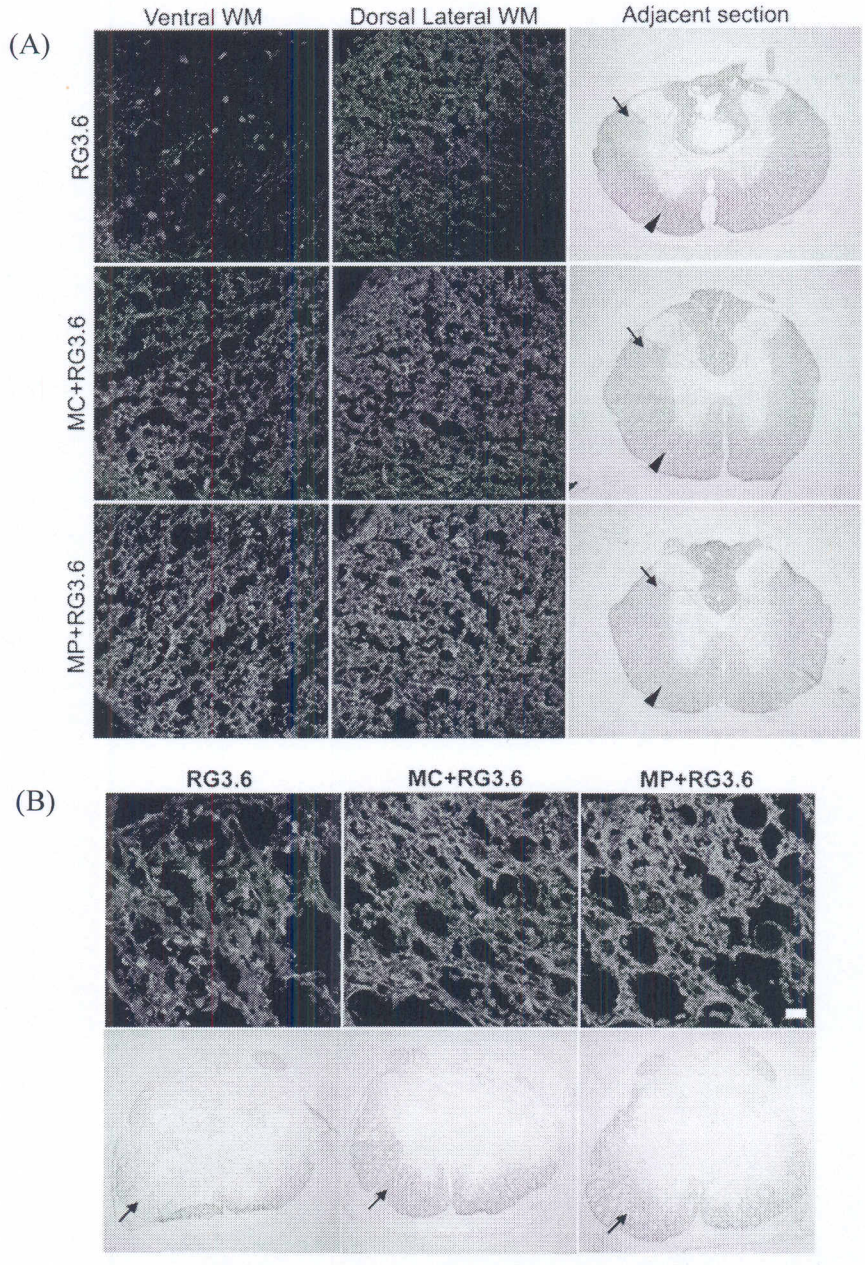


(C)



Spared white matter was stained by Luxol fast blue and quantitated by Zeiss LSM software. 1 cm segment of the spinal cord with the injury site in the center was frozen sectioned at 20 μm and every 5th section was taken. MC and MP both showed a trend to preserve more spared white matter at rostral regions, and only MC rats showed statistical significance at 1 mm rostral to lesion center (A). Spared white matter volume was reconstructed and showed that MC preserved more rostral spinal cord segment (R4-CT 4mm) than controls (B). 3D reconstruction of Luxol fast blue stained regions was obtained by Z-stack images of 2D bright-field images taken with Zeiss Axiophot and processed through 3D rendering using Imaris software (C). (R: rostral, C: caudal, and CT: lesion center. N=3 per group, data present mean ± SEM)

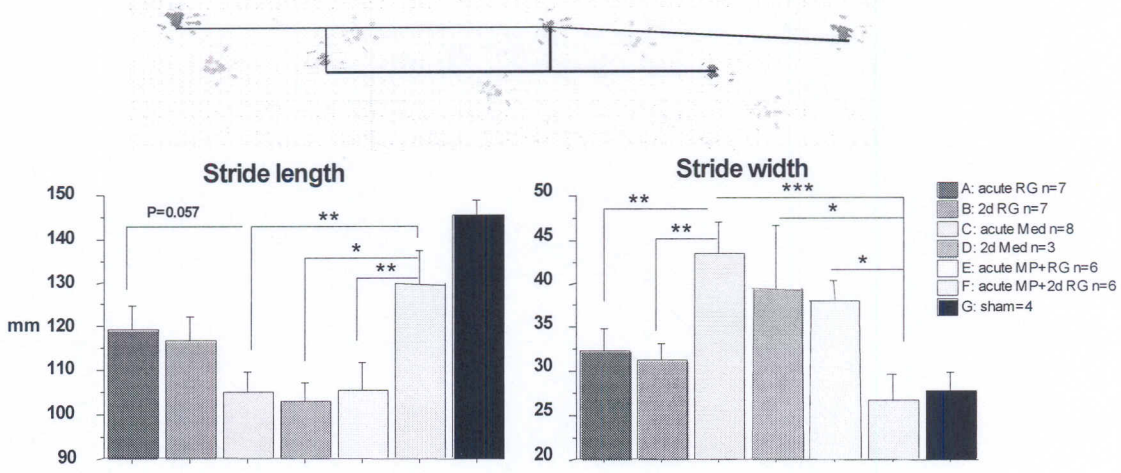
(5) Oligodendrocytes preservation



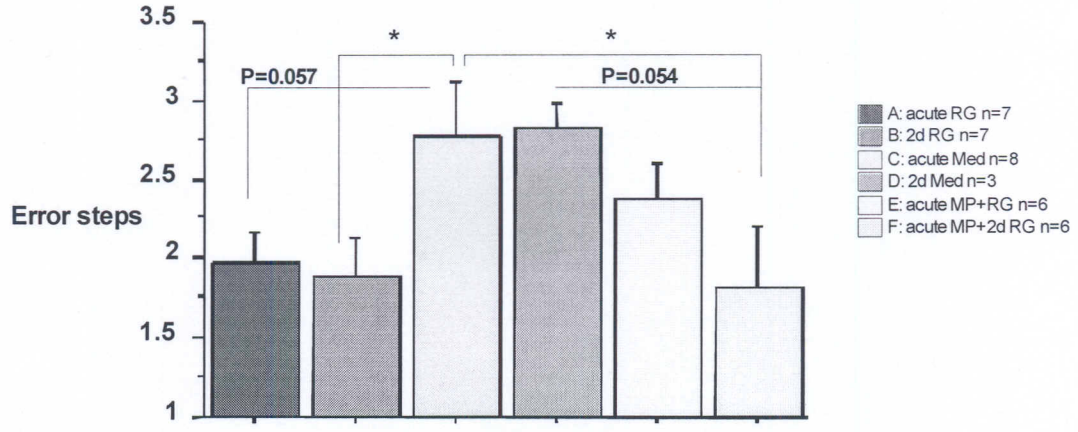
Delayed oligodendrocytes death occurs about 2 weeks after injury. We used CNPase to immunostain olidodendrocytes to determine if treatment preserve more oligodendrocytes. Rostral sections were used in (A) and showed more CNPase positive staining in the ventral white matter (WM) which is indicated by the arrow in the adjacent luxol fast blue stained section, and dorsal lateral WM by the arrowhead. Lesion centers (Shown in B) also showed more CNPase positive cells in the ventral WM. Immuostained areas were indicated by the arrow in the adjacent sections in the lower panel. Scale = 20μm.

(5) Oligodendrocytes preservation

(A) Footprint analysis



(B) Grid walking error counts



(A) Animals were placed on a 1-m long chamber, paws were inked and footprints were recorded on white paper strip. Animals with acute MP injection and sub-acute (2 d post injury) radial glia transplantation (Group F) have significant improved stepping, which is similar to naïve rats with long stride length and short stride width. Acute radial glia transplantation (Group A) also showed better stepping but this beneficial effect was reduced when combined with acute MP administration (Group E). The recovery of stepping is also improved in sub-acute RG3.6 transplantation (Group B) and was enhanced by acute MP (Group F). Animals treated with medium DMEM+F12 did not have functional recovery (Group C, D).
(B) Animals were placed on a horizontal ladder with irregular gaps and failed steps were counted. Rats received acute or sub-acute RG3.6 transplantation show significant recovery with fewest dropped steps. *P<0.05, **P<0.01, ***P<0.0001, Fisher's PLSD.