Improvement of Peripheral Regeneration with G-CSF in a Rat Model of Sciatic Nerve Repair

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Introduction:
Peripheral nerve injury can cause and lead to different degrees in the loss of functional ability. Progress in microsurgical techniques and advancements in cell therapy have improved the outcome of nerve convalescence. However, full nerve recovery is often limited and with uncertain results. To achieve a better outcome, an adjunct drug supportive therapy with induction and enhancement of nerve regeneration is strongly needed. Several cell therapy or novel technique strategies have been applied to enhance nerve regeneration. Granulocyte-colony stimulating factor (G-CSF) is a 19.6-kDa glycoprotein produced by bone-marrow stromal cells, endothelial cells, macrophages, fibroblasts, and astrocytes. It is a potent hematopoietic growth factor that mobilizes hematopoietic stem cells from bone marrow into the peripheral circulation. G-CSF has a diversity of functions, including its neuroprotective, neurogenic, anti-inflammatory, angiogenetic, and anti-apoptotic effects. Studies repeatedly have revealed the neuroprotective action and beneficial effects of G-CSF treatment following experimental cerebral and spinal cord ischemia or contusion injury. Furthermore, a few studies also have demonstrated its effects in a peripheral crush injury model. There is also a concern that only a very limited number of studies have been dedicated to microscopic nerve repair and nerve grafting. To date, however, no experimental studies have investigated the effect of GCSF on functional or histopathologic outcome after repair of a transected nerve. In this study, we investigated the effect of G-CSF administration on transected nerves that were repaired using end-to-end neurorrhaphy or nerve auto-grafting techniques. The effects of G-CSF were determined by electrophysiological test, functional neurological outcome, wet muscle weight, and histomorphological analysis.

Methods:

Experimental
Animals. We used 40 male Sprague-Dawley rats (weight: 200-250 g) in this study. The rats were randomly assigned to 1 of 4 groups with 10 rats in each group; rats in Groups I and II underwent end-to-end neurorrhaphy without and with G-CSF-treatment (100 μg/kg daily for 5 consecutive days); and rats in Groups III and IV received nerve autografting (nerve graft about 1 cm repair in retrograde fashion) without and with G-CSF-treatment, respectively, prior to surgery. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of our institution.

Electrophysiological, behavioral observation and functional assessment
The amplitude of SSEP for all groups after surgical intervention (end-to-end neurorrhaphy or nerve autografting) and the
administration of G-CSF or saline at different time points for 12 weeks are given in figures 1a and b. An overall trend towards a higher value of amplitude in comparing the end-to-end neurorrhaphy to nerve autografting, and the administration of G-CSF to saline was seen 2 to 12 weeks after operation; and was statistically significantly difference at most of the time points. An overall trend towards a lower value of latency of SSEP in comparing the end-to-end neurorrhaphy to nerve autografting, and the administration of G-CSF to saline was seen 2 to 12 weeks after operation; and was statistically significantly difference at 2nd, 4th and 12th week after operation in the end-to-end neurorrhaphy group. (Figures 2a and b). Most of all, experimental groups II and IV (end-to-end neurorrhaphy and nerve autografting treated with G-CSF) had a more significant restoration of the amplitude (Figures 3a and b) and decrease of latency (Figures 4a and b) of CMAP at every time point than groups I and III (without G-CSF treatment). All groups showed signs of recovery in the decreasing SFI from 2 to 12 weeks after operation, and demonstrated a trend similar to the electrophysiological findings (i.e., end-to-end neurorrhaphy was faster than nerve autografting, and administration of G-CSF was faster than saline). At every time point after operation, group II demonstrated a significantly higher SFI score than group I. (Figure 5a) The average SFI scores of the rats with G-CSF-treated nerve autografting (group IV) were found to be higher at 8th and 12th weeks post-operation than the rats with saline-treated nerve autografting (group III), but these differences were not statistically significant. (Figures 5b)

Results:

**Wet weight of the quadriceps and gastrocnemius**
Limb muscle atrophy was obvious in the calf muscle of the experimental limb in all groups. The percent muscle sparing after operation was analyzed using the relative ratio of the wet muscle weight of the experimental side to the control side (Fig. 6). No significant difference in the quadriceps of rats in all groups was found (range 96-100%), but there was a significant difference in G-CSF treatment between the end-to-end neurorrhaphy groups (group II vs. group I) (p < 0.01).

**Histologic observation and histomorphometrical analysis**
Figures 7 and 8 depict the morphology of nerve fibers proximally (proximal stump; A & C) and distally (distal stump; B & D) to the operated site at 12 weeks post-surgery in groups I & II and groups III & IV, respectively. Statistical comparison of the G-CSF-treated and saline-treated groups (groups III vs. IV) revealed a significant difference (p < 0.05) for mean myelin diameter at the distal stump (Fig. 9).

Discussion:

**Significance:**
Additional G-CSF treatment in microsurgical nerve repair can enhance nerve recovery and regeneration. There was also a significant improvement in functional and histological outcome with the use of G-CSF in end-to-end neurorrhaphy, and particularly in nerve autografting.

**Acknowledgments:**
Figures 2a & b. Comparison of latency change of SSEPs (femoral nerve).

Figures 2a & b. Comparison of amplitude change of SSEPs (bibial nerve).
Figures 3a & b. Comparison of amplitude change of CMAPs (gastrocnemius).

Figures 4a & b. Comparison of latency change of CMAPs (gastrocnemius).
Figures 5a & b. Comparison of the sciotic function index change.
Figure 6. Comparison of the wet muscle weight change of gastrocnemius and quadriceps between all groups.
Figure 7. The sciatic nerve was cut following end-to-end neurotomy and treated with saline (A, B) or G-CSF (C, D). The sciatic nerves were separated into proximal end (A, C) and distal ends (B, D). There were no significant morphological changes in the proximal end, but numerous myelin with small diameter in the distal end, especially in the G-CSF negative group. Scale bar 20 micrometers.
Figure 8. The sciatic nerve was cut following microsectioning and treated with saline (A, B), or G-CSF (C, D). The sciatic nerves were separated into proximal (A, C) and distal ends (B, D). There was no significant morphological change in the proximal ends. After autografting without G-CSF administration, axon loss and the spared myelin was small in diameter (B); but, otherwise, G-CSF treatment spared more myelin with a smaller medium diameter (D). Scale bar 24 micrometers.
Figure 9. The ratio of different diameter of myelin counted in each experiment group. *p < 0.05.

* Wilcoxon rank sum test, p-value < 0.05

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