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BRAIN RESEARCH

Research Report

Graft of pre-injured sural nerve promotes regeneration of corticospinal tract and functional recovery in rats with chronic spinal cord injury

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ABSTRACT

A possible treatment approach for chronic spinal cord injuries has been tested. We report that minced, autologous, pre-injured peripheral nerve administered as a single injection into an injury-induced cyst, resulting from a contusion injury of the thoracic spinal cord, stimulates recovery of hindlimb locomotor function in rats, as measured by the Basso, Beattie, Bresnahan Locomotor Rating Scale. This response was further enhanced by the addition of exogenous neurotrophic factors. Histological analysis showed axons of the corticospinal tract exhibited significant regeneration past the injury site, when quantified both by number and length. Results indicate that the use of a pre-injured peripheral nerve graft stimulates chronically injured descending nerves to overcome a local inhibitory environment. The resulting sprouting and growth past the injury site is associated with a significant improvement in locomotor function.

1. Introduction

Transplant of peripheral nerve tissue into rat spinal cord has long been known to stimulate sprouting of nerves in the central nervous system (Aguayo et al., 1981; Cheng et al., 1996; David and Aguayo 1981), but charting a clear path into the clinic for treatment for spinal cord injury has proved more challenging. Cell transplantation and the use of neurotrophic factors to stimulate regeneration of injured nerve fibres have shown promise as potential treatments for spinal cord injury (SCI), mostly in an acute setting. The choice of cell is not only restricted to cell type, but also depends on the state of the cell prior to transplantation. Thus, manipulation of cells prior to transplantation has included activation of macrophages (Rapalino et al., 1998) and culture of Schwann cells (Feng et al., 2004; Oudega and Xu, 2006) or olfactory glial cells (Cao et al., 2004; Li et al., 1997a; Sasaki et al., 2006) have been used to examine their ability to stimulate regeneration of severed axons. While Schwann cells have been a focus for transplantation studies over many years (Feng et al., 2004; Oudega and Xu, 2006), they have generally been used following culture or directly after removal from a donor animal. Furthermore, several obstacles still exist to prevent the use of Schwann cells as a therapeutic procedure for the treatment of patients with spinal cord injury. These obstacles include the lack of source of Schwann cells, difficulty of in vitro expansion, possible rejection after allografts and lack of knowledge of the time window most appropriate for the transplantation.

To promote the clinical application of Schwann cells as a therapeutic approach, in this study we have used a clinically relevant model of SCI to test whether graft tissue prepared from autologous peripheral nerve injured 7 days earlier could stimulate recovery of lost hindlimb motor function, as measured by the Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale

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(Basso et al., 1995, 1996). Given the substantial evidence that peripheral nerve distal to a lesion, expresses many known factors supportive of peripheral nerve regeneration, such as neurotrophic factors (Funakoshi et al., 1993a; Smith et al., 1993; Zhang et al., 2000), cell adhesion molecules(Gardiner et al., 2005; Weidner et al., 1999) and extracellular matrix molecules (Rezajooi et al., 2004; Siconolfi and Seeds, 2001), we reasoned that the transplantation of components of degenerating peripheral nerve might provide a stimulatory environment to facilitate the regeneration of severed descending axons of the spinal cord and assist the recovery of locomotor function.

Several studies have demonstrated the ability of transplanted, purified Schwann cells to stimulate spinal cord axon regeneration (see Bunge, 2002). In addition, peripheral nerve grafts alone or with the addition of neurotrophic factors, have also been used successfully following spinal cord injury. Furthermore, injury to peripheral nerve stimulates Schwann cell proliferation and expression of high levels of p75NTR which may play a critical role in the regeneration of peripheral nerves (Zhou and Li, 2007). As both Schwann cells and activated macrophages are two major cellular components in injured peripheral nerves, pre-injured peripheral nerve would be an ideal source for transplantation material for the treatment of chronic SCI. The sural nerve is frequently used as a suitable graft tissue for peripheral nerve repair (Namiki et al., 2007; Samii et al., 2006). Since this nerve can also provide sufficient material for transplantation, we have combined these positive aspects of the pre-injured sural nerve to examine its ability to stimulate regeneration of corticospinal axons in a manner that suitable for human use. In an earlier study (Ferguson et al., 2001) we presented axonal tracing evidence that, in a dorsal hemisection model of spinal cord injury, chronically injured cortical motor neurons retain the capacity to regenerate for extended periods of time and that regeneration can be stimulated using grafts of minced, preligated autologous peripheral nerve tissue. In this study we used a contusion spinal cord injury model to confirm regeneration of corticospinal tract past the injury site coupled with significant recovery of hindlimb motor function and this response is enhanced by adding exogenous neurotrophins.

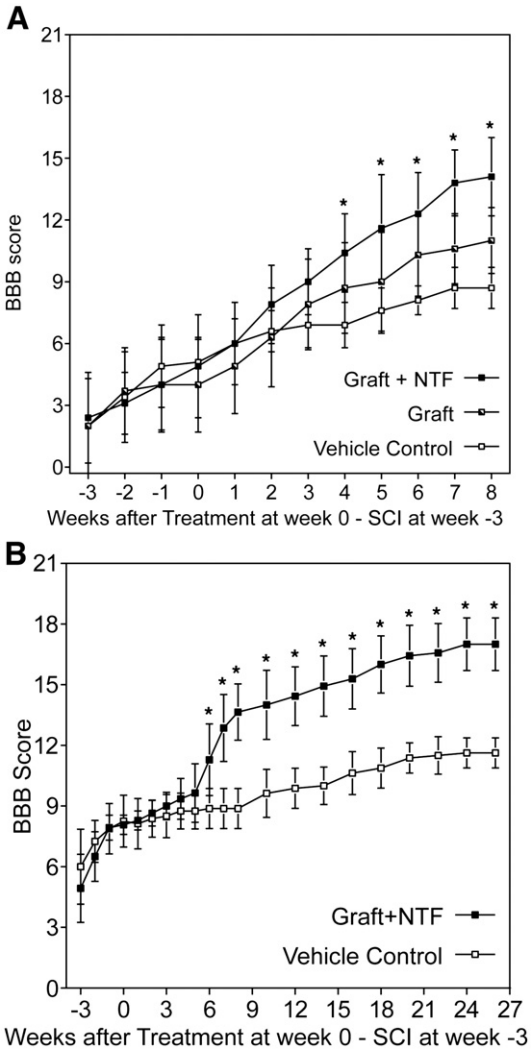

Fig. 1 – Graft and graft+NTF stimulates recovery of hindlimb locomotor function. (A) At 4 week post-treatment individual means were significantly different from group mean by one-way ANOVA and Kruskal–Wallis test. At 2 months post-treatment: vehicle control BBB score was 8.7 ± 1.0 (n = 7); graft alone BBB score was 11.0 ± 1.6 (n = 7); graft+NTF BBB score was 14.1 ± 1.9 (n = 9); each group mean significantly different from each other group mean at $P < 0.05$, independent samples t test, assuming unequal variances. (B) BBB score was 5.3 ± 1.8 (mean ± SD) on day 1 and 8.1 ± 0.83 on day 21 post-SCI. At 6 months post-treatment: vehicle control BBB score was 11.6 ± 0.55 (n = 6); graft+NTF BBB score was 17 ± 1.7 (n = 6). Graft+NTF was significantly different from vehicle control at $P < 0.001$.

2. Results

2.1. *Behaviour*

2.2.1. *The short-term study*

All animals demonstrated significant loss of motor function one week after SCI, having an average BBB score between 2 and 3 at the first test (Fig. 1A). The hindlimbs of animals were paralyzed with a variable, slight to extensive joint movement. Three weeks after treatments, motor function was improved in all groups of animals with no significant difference being seen between the groups (Fig. 1A). At later times after treatment, motor function continued to show improvement even in vehicle treated rats. Although the BBB scores in the graft alone group were slightly higher than for the vehicle control group, these differences did not reach statistical significance. In contrast, BBB scores in the graft plus neurotrophic factors (NTF) group significantly improved over the second month after treatment (Fig. 1A). The animals in the combined graft plus NTF group could walk with weight bearing, whereas most of vehicle treated animals dragged their hindlimbs and none were able to walk with limb coordination.

2.2.2. *The long-term study (Fig. 1B)*

Immediately after SCI, all animals displayed hindlimb paralysis with slight joint movement. One week after injury, movement was significantly improved in all animals. Three weeks after SCI,