INTRODUCTION

The adult central nervous system has a limited capacity for axonal regeneration following injury. Certain types of central neurons, however, are able to regenerate if a permissive environment is provided. One such environment can be achieved with transplanted Schwann cells (1). Previous experiments have shown compressive mechanical stress to be important in stimulating the regenerative behavior of Schwann cells (2,3). The purpose of this project is to study how pre-conditioned Schwann cell transplants may promote axonal regeneration and functional recovery of spinal cord injured tissue. Furthermore, this proposal uses a new surgical approach for harvesting and transplanting the pre-conditioned Schwann cells which may eventually offer a therapeutic alternative for the human spinal cord injured (SCI) patient.

METHODS

Our donor tissue consisted of Sprague-Dawley rat sciatic nerve compressed for two weeks via atrumatic implantation of silastic tubing. The contralateral (control) sciatic nerve in each rat was also exposed and mobilized, but returned to its host bed with minimal trauma. Recipient rats all underwent a laminectomy at T10 followed by a hemisection of its dorsal column at the same level. The dorsal hemisection model was created with a Feather MicroScalpel Feather 15º (EMS, Hatfield, PA). The donor sciatic nerve segment was harvested and immediately sutured into the injured spinal cord cavity using microsurgical technique. Functional outcome was measured using the Basso, Beattie, Bresnahan (BBB; 4) Locomotor Rating Scale at weekly intervals including preoperatively, one week post-injury, and weekly thereafter for six weeks post-surgery. Stereotactic injections of biotinylated dextran amine- BDA (Molecular Probes, Eugene, Oregon) were performed via a craniotomy over the rat motor cortex to verify axonal regeneration in the descending tracts in a few animals. Ascending spinal cord tracts were evaluated in the remaining rats with intraneural injection of BDA to the L4 dorsal root ganglion. After tract tracing, the animals were allowed to survive for two additional weeks, following which they were perfused with 4% paraformaldehyde (PFA). Intact spinal cords were harvested and post-fixed in 4% PFA, rinsed in Na 2HPO 4, equilibrated in 30% sucrose buffer, and embedded in TissueTek® (VWR International, West Chester, PA). The frozen tissue blocks were then sectioned to produce longitudinal sections of the lesion site, and cross-sections of the rostral and caudal segments. Visualizing the BDA-labeled axons was possible through histochemical means using avidin and biotinylated horseradish peroxidase (Vectastain ABC Kit, Vector Labs, Burlingame, CA) followed by diaminobenzidine (DAB) staining, allowing for dark staining of axons along with light staining of the gray matter (5).

RESULTS

Functional analysis of recovery with BBB scores reflects a modest improvement in function after transplanting pre-conditioned peripheral neural tissue in the later time points (>4 weeks) that was not present in the early post-operative period (Table 1). Both transplanted groups had greater recovery than the non-transplanted group with the pre-conditioned transplant group having the highest recovery overall.

Following confirmation that the descending spinal cord tracts were labelled with BDA, evaluation of longitudinal specimens revealed numerous axons labeled in the rostral segment (Fig. 2) along with a mild sprouting at the transplant area (Fig. 3). Histological analysis demonstrated more pronounced axonal sprouting within the pre-conditioned transplant groups than in non-conditioned transplant group.

CONCLUSIONS

This study provides stimulating new data about a new therapeutic approach for SCI. Histological analysis revealed an increase in axonal sprouting/regeneration. Furthermore, functional recovery improved following the transplant of pre-conditioned peripheral neural tissue into the injury site. Although the functional recovery did not significantly differ in the early post-operative periods, there was a significant trend in recovery later. The promising histologic results with ensuing modest functional improvements suggest further investigation is warranted to ascertain if the increased axonal regeneration after treatment will translate into greater functional improvements at later timepoints.

 REFERENCES


Acknowledgements: Funding from NIH/NINDS NS02221 & NS049203 and the Roman Reed Foundation.