Adipose Derived Stem Cell Transplantation Does Not Improve Recovery of Motor Function after Sciatic Nerve Transaction in Rats

D. Angius¹, A.M. Knight², R.J. Spinner¹, M.J. Yaszemski³, A. J. Windebank².

wondebank.anthony@mayo.edu

¹ Department of Neurosurgery; ² Department of Neurobiology; ³ Department of Orthopaedics; Mayo Clinic, Rochester USA

Introduction

In the peripheral nerve system (PNS), the challenge is to develop an alternative to the autologous nerve graft and thus reduce the necessity of two surgical procedures and the harvesting of tissue from the patient. Also, clinical functional recovery rates typically reach only 80% for nerve injuries treated using autologous nerve grafts. Thus, bioengineering techniques for the PNS have focused on developing alternative treatments to the nerve graft (e.g., nerve guidance scaffolds), in particular for larger defects, and improving recovery rates and functional outcome. We synthesized a series of single-lumen crosslinked poly(e-caprolactone) fumarate (PCLF) nerve conduits. Based on the hypothesis that cell transplantation in a bioartificial conduit is an alternative strategy to create a favourable environment for nerve regeneration, we loaded the nerve scaffolds with adipose derived stem cells. Regeneration in the experimental group was compared to control groups using electrophysiology, neuromorphometry and proteomic analysis as assessments.

Materials and Methods

Adipose derived stem cells (ADSCs) were isolated from adult Lewis rats anesthetized after harvesting of subcutaneous adipose tissue. The fat was carefully dissected, minced and then enzymatically dissociated. The solution was processed and centrifuged, and the stromal cell pellet was resuspended in MEM containing 20% (v/v) FBS and 1% (v/v). Cultures were maintained at sub-confluent levels in a 37°C incubator until transplantation. The crosslinked single lumen PCLF conduits were fabricated and sterilized in 80% ethanol solution for 30 min and dried under vacuum overnight before they were implanted to graft a 1cm gap in a rat sciatic nerve model. In the experimental group the nerve scaffolds were filled with 1x10⁶ ADSCs. The sciatic nerve was either crushed by applying maximal force with a smooth forceps for 10 s, or transected with sharp microscissors and grafted by an autologous graft, a PCLF tube filled with ADSC or saline solution. Each experimental group included 16 Lewis rats. The nerve repair was evaluated 16 weeks after implantation. Regeneration capacity was analyzed with compound muscle action potential (CMAP) recordings and quantitative nerve morphometry. Qualitative analysis was performed by investigating differences in protein profiles in each experimental group.

Results

The quantitative analysis of axonal regeneration showed that the CMAP amplitudes were significantly better in the autograft and crush injury groups compared with normal. After repair using a PCLF scaffold loaded with saline or with ADSCs, there were significantly less myelinated fibers if compared to the crush injury, autograft and normal group. The myelinated fibers were also smaller and less myelinated in all the experimental groups compared to the normal control. The proteomic studies have provided better pictures of cellular and molecular changes in the degenerating nerve and its microenvironment after sciatic nerve injury showing that several proteins were significantly over and under-expressed in different models of nerve injury. Since there are apparent behavioral and morphological differences among the most utilized animal neuropathy models, comparison of protein expression patterns from different types of injuries helped us understand elaborate molecular and cellular mechanisms underlying diverse peripheral neuropathy and peripheral nerve regeneration.

Discussion and Conclusions

The present study did not show any beneficial effect following entubulation with adipose derived stem cells. Compared to control treatment with a PCLF scaffold alone, entubulation with ADSCs failed to improve the extent axonal regeneration at the lesion site and did not improve functional recovery.

Acknowledgement: AFIRM Consortium W81XWH-08-2-0034; Mayo Foundation.

Disclosures

No conflict of interest disclosure information in this work.