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Hydrophilic polymers enhance early functional outcomes after nerve autografting

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ABSTRACT

Background: Approximately 12% of operations for traumatic neuropathy are for patients with segmental nerve loss, and less than 50% of these injuries obtain meaningful functional recovery. Polyethylene glycol (PEG) therapy has been shown to improve functional outcomes after nerve severance, and we hypothesized this therapy could also benefit nerve autografting.

Methods: We used a segmental rat sciatic nerve injury model in which we repaired a 0.5-cm defect with an autograft using microsurgery. We treated experimental animals with solutions containing methylene blue (MB) and PEG; control animals did not receive PEG. We recorded compound action potentials (CAPs) before nerve transection, after solution therapy, and at 72 h postoperatively. The animals underwent behavioral testing at 24 and 72 h postoperatively. After we euthanized the animals, we fixed the nerves, sectioned and immunostained them to allow for quantitative morphometric analysis.

Results: The introduction of hydrophilic polymers greatly improved morphological and functional recovery of rat sciatic axons at 1–3 d after nerve autografting. Polyethylene glycol therapy restored CAPs in all animals, and CAPs were still present 72 h postoperatively. No CAPS were detectable in control animals. Foot Fault asymmetry scores and sciatic functional index scores were significantly improved for PEG therapy group at all time points ($P < 0.05$ and $P < 0.001$; $P < 0.001$ and $P < 0.01$). Sensory and motor axon counts were increased distally in nerves treated with PEG compared with control ($P = 0.019$ and $P = 0.003$).

Conclusions: Polyethylene glycol therapy improves early physiologic function, behavioral outcomes, and distal axonal density after nerve autografting.

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1. Introduction

Axonal fusion has been reported as a natural mechanism used by invertebrates to promote rapid and highly specific neuronal recovery of complex behaviors after injury [1,2]. Hydrophilic polymers such as polyethylene glycol (PEG) have also been shown to rejoin the axolemma of the cut ends of severed proximal and distal axons, inducing both morphological and functional axonal continuity (PEG fusion) [3–6]. Scientists have been using PEG for decades to fuse cells to immortalize desired cell lines such as monoclonal antibody–producing B-cells [7].

Polyethylene glycol facilitates lipid bilayer fusion by removing water from the lipid bilayer at or near the damage site, decreasing the activation energy required for plasmalemmal leaflets to fuse [8,9]. These same properties of PEG help to restore functional connections between proximal axonal segments connected to their cell bodies and distal severed axons [3–6,10]. Specifically, within 1–2 min after cut or crush severance, PEG has been used *in vitro* to rapidly reconnect the severed proximal and distal halves of individually-identified invertebrate giant axons as measured by intra-axonal dye diffusion [10].

Most recently, PEG has been used to rapidly restore axonal morphological and physiological continuity of cut- or crushed- severed mammalian sciatic nerves *in vitro*, *ex vivo*, and/or *in vivo* [3–6,11,12]. Recent studies have also demonstrated that PEG has a neuroprotective effect after acute spinal cord injury in the rat model [5,13]. However, no study has been performed using PEG combined with other procedures to restore morphologic or physiologic continuity after segmental nerve loss treated with nerve autografting. If successful, the ability to fuse severed nerves with exogenous application of PEG and regain rapid functional recovery has the potential to produce a paradigm shift in therapeutic management after peripheral nerve damage.

Sealing of axolemmal damage normally occurs through a calcium-dependent accumulation of membranous structures that interact with nearby undamaged membrane to form a seal [14–16]. Calcium also initiates processes leading to cell death and axonal Wallerian degeneration (breakdown of the axon distal to the site of injury) within 48–96 h after injury [9,17]. In severed nerves, this calcium-dependent system for plasmalemmal repair seals the cut ends of partially collapsed axons with vesicles, preventing them from possibly fusing with an adjacent open axonal stump [5,6].

Our protocol includes irrigating the site of nerve injury before PEG fusion in a calcium-free, isotonic, isosmotic solution to open axonal ends and remove vesicles. The antioxidant methylene blue (MB) is also added before adding PEG, to reduce vesicle formation [5]. After adding PEG to induce PEG fusion of open, vesicle-free axonal ends, the PEG is washed away by isotonic saline containing calcium so that vesicles form to seal any remaining holes [3–6,9,14,15]. We hypothesize that this therapy will restore nerve electrophysiology after interposition autografting, and lead to improved behavioral outcomes.

2. Materials and methods

All experimental procedures were approved by and performed in accordance with the standards set forth by the Institutional Animal Care and Use Committee at Vanderbilt University.

2.1. *Surgical procedures*

We anesthetized Female Sprague-Dawley rats with inhaled isoflurane, shaved the left hind limb with clippers, and prepped it aseptically. We made a 2-cm incision parallel and just caudal to the femur. Using sharp dissection, we freed the cephalad border of the biceps femoris to allow for caudal retraction and exposure of the left sciatic nerve. This exposure allows for visualization of the entire sciatic nerve without the need for muscle division. We then dissected the exposed nerve free of perineural tissue using sharp dissection and minimal retraction. We bathed the exposed nerve in PlasmaLyte-A (Baxter: Deerfield, IL) and performed electrophysiological testing. PlasmaLyte-A is a calcium-free solution containing the following (in mEq/L): Na 140, K 5, Mg 3, Cl 98, acetate 27, gluconate 23. The solution is at pH 7.4 and contains 294 mOsm/L.

We then removed a 5-mm segment of sciatic nerve and irrigated the wound with PlasmaLyte-A. Using standard microsurgical techniques, with careful attention paid to maintain orientation, we sutured the removed segment of sciatic nerve in place using 9–0 Ethilon (Ethicon, Somerville, NJ). Once we approximated both ends in an end-to-end fashion using an interrupted suturing technique and microscopic magnification, we applied a hypotonic 1% by weight solution of MB (Acros Organics, Morris Plains, NJ) in sterile water to the coaptation sites for 1 min. We then applied a 50% by weight solution of PEG (3.35 KD molecular weight; Sigma-Aldrich, St. Louis, MO) in sterile water to the coaptation sites for 1 min in experimental animals. Control animals received only the MB solution.

We then irrigated the wound with Lactated Ringer's solution (Hospira, Lake Forest, IL) and repeated electrophysiological testing. Lactated Ringer's solution contains the following (in mEq/L): Na 130, K 4, Ca 2.7, Cl 109, and lactate 28. This isotonic, calcium-containing solution is at pH 6.5 and has 273 mOsm/L. We approximated the skin using a running subcuticular 5–0 Monocryl suture (Ethicon). We then gave all control and experimental animals a subcutaneous injection of ketoprofen (5 mg/kg) and allowed them to emerge from anesthesia. Ketoprofen is almost completely excreted by 24 h postoperatively and has minimal, if any, impact on behavioral testing occurring after that time [18].

At 72 h postoperatively, after behavioral testing had been completed, we again anesthetized the rats with inhaled isoflurane and prepared the left hind limb as previously described. Using the same exposure technique, we exposed the left sciatic nerve. We irrigated the wound with PlasmaLyte-A and repeated electrophysiological testing.

We then euthanized the rat via intracardiac injection of Fatal-Plus Solution (Vortech, Dearborn, MI). We harvested