SCIENTIFIC ARTICLE

Evaluation of a Nerve Fusion Technique With Polyethylene Glycol in a Delayed Setting After Nerve Injury

Ravinder Bamba, MD,*† David Colton Riley, BS,*‡ Justine S. Kim, BS,* Nancy L. Cardwell, BS,* Alonda C. Pollins, MLI,* R. Bruce Shack, MD,* Wesley P. Thayer, MD, PhD*

Purpose Polyethylene glycol (PEG) has been hypothesized to restore axonal continuity using an *in vivo* rat sciatic nerve injury model when nerve repair occurs within minutes after nerve injury. We hypothesized that PEG could restore axonal continuity when nerve repair was delayed.

Methods The left sciatic nerves of female Sprague-Dawley rats were transected and repaired in an end-to-end fashion using standard microsurgical techniques at 3 time points (1, 8, and 24 hours) after injury. Polyethylene glycol was delivered to the neurorrhaphy in the experimental group. Post-repair compound action potentials were immediately recorded after repair. Animals underwent behavioral assessments at 3 days and 1 week after surgery using the sciatic functional index test. The animals were sacrificed at 1 week to obtain axon counts.

Results The PEG-treated nerves had improved compound action potential conduction and animals treated with PEG had improved sciatic function index. Compound action potential conduction was restored in PEG-fused rats when nerves were repaired at 1, 8, and 24 hours. In the control groups, no compound action potential conduction was restored when nerves were repaired. Sciatic functional index was superior in PEG-fused rats at 3 and 7 days after surgery compared with control groups at all 3 time points of nerve repair. Distal motor and sensory axon counts were higher in the PEG-treated rats.

Conclusions Polyethylene glycol fusion is a new adjunct for nerve repair that allows rapid restoration of axonal continuity. It effective when delayed nerve repair is performed.

Clinical relevance Nerve repair with application of PEG is a potential therapy that may have efficacy in a clinical setting. It is an experimental therapy that needs more investigation as well as clinical trials. *(J Hand Surg Am. 2017;■(■):1.e1-e7. Copyright © 2017 by the American Society for Surgery of the Hand. All rights reserved.)*

Key words Axonal fusion, microsurgery, nerve injury, nerve repair, polyethylene glycol.

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POLYETHYLENE GLYCOL (PEG) IS A hydrophilic polymer that can rejoin the axolemma of the cut ends of severed axons, inducing partial axonal continuity in a process known as PEG fusion.[1–4] It has been hypothesized that PEG facilitates lipid bilayer fusion by removing water near the injury site, decreasing the activation energy required for plasmalemmal leaflets to fuse.[5,6] Polyethylene glycol has been

*From the *Department of Plastic Surgery, Vanderbilt University Medical Center, Nashville, TN; and the †Department of Surgery, Georgetown University, and ‡Georgetown University School of Medicine, Washington, DC.*

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Corresponding author: Ravinder Bamba, MD, Department of Plastic Surgery, Vanderbilt University Medical Center North, 1161 21st Avenue S, S-2221, Nashville, TN 37232; e-mail: rbamba3@gmail.com.

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used as a membrane fusogen to create hybridomas, to immortalize monoclonal antibody-producing B cells.[7] With its membrane-fusing properties, PEG has been investigated *in vitro*, *ex vivo*, and *in vivo* in invertebrates and vertebrates to determine whether PEG had the ability to fuse axons.[1,8–11] Polyethylene glycol fusion has been found to restore axonal continuity partially in a rat sciatic nerve injury model.[5]

The effect of PEG fusion in nerve repair is thought to result from the prevention of axolemmal sealing, approximation of axonal endings, and application of PEG directly to the neurorrhaphy site. After neurotemesis, the sealing of axolemma typically occurs via a calcium-dependent accumulation of membranous structures that interact with nearby undamaged membrane to form a seal. In severed nerves, the calcium-dependent mechanism for plasmalemmal repair seals the cut ends of partially collapsed axons with vesicles, preventing them from possibly fusing with an adjacent open axonal stump. Our method for PEG fusion includes irrigating the nerve injury site with a calcium-free saline solution to prevent axolemmal sealing. After nerve repair, a PEG solution is applied to induce fusion of open, vesicle-free axonal ends. Polyethylene glycol is subsequently washed away with a calcium-containing saline solution to induce vesicle formation to seal any remaining holes.[5]

Polyethylene glycol fusion has shown superior outcomes after neurotemesis with direct repair as well as after nerve gap repairs with allografts and autografts in a rat sciatic nerve model.[2–4] Outcomes have been traditionally measured by postoperative compound action potentials (CAPs), behavioral data, and total axon counts. However, in these studies, nerve repair occurred within minutes after nerve transection. Because nerve repairs are rarely performed this soon after injury, questions of the clinical translation of PEG fusion have arisen.

The purpose of this study was to investigate the efficacy of PEG fusion in a less immediate setting after nerve injury in a rat sciatic nerve model. A delayed setting for nerve repair after transection is more reflective of clinical practice than immediate repair, and investigation into time limitations of PEG fusion will provide more insight into the clinical translation potential of PEG fusion. We hypothesized that PEG would improve outcomes when nerve repair was performed in a less immediate setting.

MATERIALS AND METHODS

Experimental animals

Adult female Sprague-Dawley rats (250 g, Charles River Laboratories, Wilmington, MA) were used in this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Vanderbilt University Medical Center.

Experimental design

Thirty female Sprague-Dawley rats were divided into 6 groups. A sciatic nerve injury model was used and all rats underwent complete nerve transection with a delayed nerve repair at 1 of 3 different time points (1, 8, or 24 hours). The times were chosen out of convenience and to make the study feasible. The authors of this study recognized that nerve repairs within 24 hours are still considered acute repairs. This study is a starting point to examine the time restraints on nerve repair using PEG. The sample size was calculated for a power of 80% to detect a difference of 200 axons among groups. The sample size calculation to detect a difference of 200 axons was based on our laboratory's previous studies, which detected an approximately 200 axon count difference between PEG-treated nerves and controls. We powered the study to determine whether a similar axon count differential existed.[2,4,12]

Surgical procedures

After the animals inhaled anesthesia, the left sciatic nerve was exposed as previously described.[3] The nerve was irrigated with PlasmaLyte-A (Baxter, Deerfield, IL), a calcium-free solution. Electrophysiological testing was performed to obtain preinjury CAP conduction recordings.

A complete transection of the left sciatic nerve was made; the wound was irrigated with PlasmaLyte-A and closed. The animals were then allowed to emerge from anesthesia.

The animals were monitored for their respective group's time interval (1, 8, or 24 hours). After the allotted time, the animals were reanesthetized and the wound was reopened. The nerve endings were resected back flush with the epineurium and were irrigated with PlasmaLyte-A. A hypotonic solution, 1% by weight, of methylene blue (Acros Organics, Morris Plains, NJ) in sterile water was applied to both nerve ends. Using microsurgical technique, we performed a standard end-to-end nerve repair using 9-0 nylon (Ethicon, Somerville, NJ). Once the nerve was reapproximated, 1% methylene blue was applied to the nerve repair site. We then applied a solution, 50% by weight, of PEG (3.35 kD molecular weight, Sigma-Aldrich, St. Louis, MO) to the coaptation sites for 1 minute in experimental animals. The control animals received all listed solutions except for PEG.