Polyethylene Glycol Rapidly Restores Physiological Functions in Damaged Sciatic Nerves of Guinea Pigs

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- OBJECTIVE: We have studied the ability of the hydrophilic polymer polyethylene glycol (PEG) to anatomically and physiologically reconnect damaged axons of the adult guinea pig spinal cord. Here we have extended this approach to test whether completely severed guinea pig sciatic nerves in isolation could be fused and whether PEG was able to repair severe standardized crush injuries to sciatic nerves in vivo.
- METHODS: The fusion test was performed with isolated sciatic nerves maintained in a double-sucrose gap recording chamber. For in vivo experiments, the sciatic nerve was surgically exposed in the hind leg of deeply anesthetized adult guinea pigs and was crushed proximal to its insertion in the gastrocnemius muscle. PEG was injected just beneath the epineurium with a 29-gauge needle, allowed to remain in the damaged axon region for 2 minutes, and removed. Sham-treated guinea pigs received an injection of water or Krebs' solution. Three indices of recovery were simultaneously monitored in response to electrical stimulation of the proximal nerve, i.e., 1) recovery of compound muscle action potentials (in millivolts), 2) contraction force of the muscle (in dynes), and 3) displacement of the muscle (in millimeters).
- **RESULTS:** When isolated sciatic nerves were severed within the double-sucrose gap chamber, compound action potential propagation through the transection plane was eliminated. After abutment of the two segments and 2-minute PEG application to this site, variable compound action potential recovery was measured in all four cases. The crush injuries to the sciatic nerve in vivo eliminated the three functional responses to sciatic nerve stimulation in all animals. Within the first 30 minutes after treatment, only 1 of 12 control animals exhibited spontaneous recovery in any of these measures, compared with six of eight PEG-treated animals. By 45 minutes, two more sham-treated animals and one more PEG-treated animal had recovered at least one functional response. This difference in proportions between PEG-treated and sham-treated animals was statistically significant ($P \le 0.02$).
- **CONCLUSION:** We conclude that these preliminary data suggest that PEG application may be a way to interfere with the steady dissolution of peripheral nerve fibers after mechanical damage and to even functionally fuse or reconnect severed proximal and distal segments. (Neurosurgery 50:147–157, 2002)

Key words: Axon sealing, Axotomy, Nerve injury, Neurotrauma, Peripheral nerve, Polyethylene glycol

estruction of nervous tissue as a result of mechanical damage is delayed but progressive. If the focal compression is severe enough, then the resultant breaches in the axolemma lead to ionic derangement, progressive dissolution of the membrane and axoplasm, and axotomy. The initial insult in all cases, including anoxic/ischemic injury, is a failure of the membrane to properly maintain ionic differences (principally Ca²⁺ ions) across itself (3, 7, 9, 12, 15, 26). This "occult" damage is thought to initiate or facilitate numerous biochemical changes, including up-regulation of lipid peroxidation metabolism and the formation of highly reactive

oxygen metabolites (so-called free radicals) (9, 26). This progressive localized pathological process is somewhat balanced by endogenous sealing of the membrane. However, if the insult is severe, then secondary axotomy is the likely result. In mammals, loss of the distal axonal segment deafferentates target cells and tissues, producing functional deficits and even irreversible loss of these targets, many of which are dependent on innervation for their integrity.

There are two basic approaches to the inhibition of progressive destruction in the nervous system caused by severe acute insults. One approach is to protect nerve cells and their pro-

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cesses from continuing destruction by blocking the biochemical processes known to contribute to it. Examples of such "neuroprotection" strategies include acute application of free radical scavengers, as well as blockade of the receptors for endogenous toxins liberated from cells in the injury region (9, 26). We have been interested in another approach, in which the cell membrane "lesions" are rapidly physically repaired, leading to very swift recovery of conduction and behavioral functioning. This is accomplished by topical application of the hydrophilic polymer polyethylene glycol (PEG). PEG has been known for more than 25 years to fuse the membranes of closely abutted cells; PEG and polymers with similar capabilities are sometimes referred to as "fusogens" (18, 25). We have demonstrated that a 2-minute application of PEG can immediately reconnect completely severed strips of guinea pig spinal cord white matter, allowing recovery of compound action potential (CAP) propagation through the plane of transection within minutes (25). This physiological recovery is associated with restored anatomic integrity of the transected axons. We have also demonstrated that PEG can restore physiological functioning within minutes in severely crushed spinal cord and that this is associated with sealing of membrane breaches large enough to allow passage of a large label, i.e., horseradish peroxidase (24).

Here we first tested the ability of PEG application to fuse severed axons of the isolated sciatic nerve, permitting immediate recovery of CAP conduction, using our previously described recording techniques. A brief application of PEG proved to be able to reconnect the proximal and distal sciatic segments; therefore, we next attempted to reverse functional loss subsequent to a severe crush injury of the sciatic nerve in an in vivo sciatic injury model. We demonstrate that PEG can produce statistically significant enhancement of muscle functioning after mechanical damage to the motor efferent, compared with sham-treated animals, which displayed variable levels of endogenous repair. We further discuss the clinical significance of this work.

MATERIALS AND METHODS

Removal of sciatic nerves for in vitro studies

The sciatic nerves of adult female guinea pigs (body weight, 350–500 g) were used for these experiments. The animals were deeply anesthetized with 60 mg/kg ketamine hydrochloride and 10 mg/kg xylazine, administered intramuscularly before dissection of the sciatic nerve. After adequate anesthesia had been achieved, the sciatic nerve was dissected from its exit from the sciatic notch of the hind leg to beyond its branching into the tibial and peroneal nerves. After the sciatic nerve had been exposed, it was gently worked free from the underlying fascia with a blunt probe. An approximately 38-mm length was removed to an oxygenated vial of Krebs' solution, after severing of the nerve at its proximal and distal ends. All animal use was in compliance with state, federal, and university guidelines, under protocols approved by the Purdue University Animal Care and Use Committee.

In vitro sciatic fusion and electrophysiological recordings

The isolated sciatic nerves were placed in a threecompartment, double-sucrose gap recording chamber (*Fig. 1*). Full descriptions of this chamber, including diagrams and details of its construction and use, were previously reported (22, 23, 25). Briefly, approximately 38-mm-long segments of sciatic nerve were placed in the chamber, crossing all of its three large interconnected compartments. The ends of the nerve were immersed in isotonic KCl (120 mmol/L), whereas the central region was immersed in Krebs' solution (124 mmol/L NaCl, 2 mmol/L KCl, 1.24 mmol/L KH₂PO₄, 1.3



FIGURE 1. Double-sucrose gap isolation chamber. The five-compartment chamber is shown, and its overall dimensions are indicated. The segment of sciatic nerve was placed as shown, traversing all compartments. A flow of medium through the sucrose solution- and Krebs' solution-containing compartments was maintained by constant aspiration during the course of each experiment. Small pieces of greased coverslips were inserted on both sided of each sucrose chamber, to further reduce mixing of media. The distance between the stimulation (first of the pair) and recording electrodes was approximately 36 mm.

mmol/L MgSO₄, 26 mmol/L CaCl₂, 10 mmol/L sodium ascorbate, 10 mmol/L dextrose, 26 mmol/L NaHCO₃, equilibrated with 95% O₂/5% CO₂). Therefore, the ends were maintained at approximately intracellular potential, whereas the middle of the sciatic nerve was maintained at approximately extracellular potential. These three large compartments were separated by small compartments of flowing sucrose (230 mmol/L), which helped to maintain electrical isolation of the ends of the nerve and to reduce mixing of the media. CAPs were evoked by bipolar electrodes at one end and recorded at the other end of the strip of spinal cord continuously during each experiment. Recordings were initiated after the nerve had equilibrated within the chamber and continued during and after complete transection of the sciatic nerve within the middle compartment.

For typical physiological functioning of the nerve, an incubation period of approximately 30 to 60 minutes, with immersion in oxygenated Krebs' solution at 37°C, was required. After CAP propagation had stabilized, the sciatic nerve was completely severed with a laboratory-fabricated cutter (a razor blade shard attached to an applicator stick); the two ends of the nerve were observed, with a stereomicroscope, to be separated by approximately 1 mm. Stimulation and recording were continued during transection, which completely eliminated the conduction of CAPs from one end to the other. After transection, the two ends of the cord segments were pushed together, i.e., abutted tightly by using a laboratory-fabricated device that applied gentle pressure on one segment of the sciatic nerve, pressing and holding it against the other segment (25). The device was mounted on a micropositioner and contacted the spinal cord parenchyma with a strip of nylon mesh stretched across two metal bands (25). A solution of M_r 1800 PEG (50%, by weight, in distilled water) was applied to the abutted segments by pressure injection through a micropipette, as a continuous stream approximately 0.5 mm wide and continuing for approximately 2 minutes. The PEG was applied to one side of the transection, washed across it, and removed on the other side by aspiration with a second suction pipette. During the PEG application, a continuous stream of oxygenated Krebs' solution was maintained. The electrophysiological properties of the fused sciatic nerve were continuously monitored for approximately 1 hour. The storage of real-time digitized physiological data, management of these data, and signal averaging of elicited CAP waveforms were accomplished by using a custom-designed LabVIEW computer program (National Instruments Corp., Austin, TX) on a Power Macintosh G-3 computer (Apple Computer, Inc., Cupertino, CA).

In situ isolation of sciatic nerves

For in situ experiments, the sciatic nerve of the hind leg was surgically exposed past its distal branches, as described above. The skin was incised and dissected away from the gastrocnemius muscle (*Fig. 2*). The dorsolateral aspect of the entire gastrocnemius muscle and its distal insertion (Achilles' tendon) was exposed. All branches of the sciatic nerve except that to the gastrocnemius muscle were incised with iris scissors. Care was taken to frequently irrigate the entire exposed wound with lactated Ringer's solution during dissection and



FIGURE 2. In situ experimental configuration. This drawing shows the surgical exposure of the sciatic nerve, its branchs (which are cut; see Materials and Methods), and the gastrocnemius muscle. The position of the two transducers, one measuring the force of muscle contraction and the other measuring the displacement of the hind paw, should be noted. The relative position of the hook electrodes stimulating the sciatic nerve proximal to its insertion in the gastrocnemius muscle is indicated, as is the placement of bipolar disc electrodes on the muscle to record the spread of CAPs in response to stimulation. The inset emphasizes that all records were acquired simultaneously on three channels; the fourth was used to dis-

play an event marker triggered by the stimulation pulse. For descriptive purposes, the drawing is not to scale.

physiological recording, to avoid desiccation. After exposure of the sciatic nerve and gastrocnemius muscle, the animal was secured to a Plexiglas platform, with the pelvis and lower limbs elevated approximately 3 cm above the station. The elevation of the limbs enabled free passive and active ankle motion.

Electrophysiological recording in situ

Hook-shaped Ag/AgCl stimulation electrodes were fabricated from 26-gauge silver wire and, with a micropositioner, gently supported the proximal sciatic nerve just as it exited the sciatic notch. Petroleum jelly was applied to the contact area to cover it and help insulate the point of electrical stimulus from the rest of the body. A paddle-shaped transducer (LAB FT-100; CB Science, Inc., Dover, NH) was positioned with a micropositioner so that the paddle was firmly against the distal metacarpal bones of the same foot. This transducer was calibrated to measure the force of contraction of the gastrocnemius muscle in dynes. One end of a sliding displacement transducer (LAB DT-475; CB Science, Inc.) was attached to the table, and the slide bar was sutured to the Achilles' tendon with 3-0 silk sutures. With this arrangement, the displacement of the muscle (in millimeters) was measured during contraction. Finally, a pair of Ag/AgCl disc electrodes were fabricated from 20-gauge wire, by heating the end of the bare silver wire to a molten state and pressing the tip to ice. These "disc electrodes" were then chloriodized by conventional techniques. The pair was placed on the belly of the gastrocnemius muscle, with electrode spacing of approximately 2 to 3 mm, to measure CAPs (in millivolts) after electrical stimulation of the sciatic nerve (Fig. 2).

After the application of all electrodes and transducers, the sciatic nerve was stimulated with square-wave pulses (≤ 6 Hz, 1-ms duration) from the integral stimulator of the LAB computer-managed integrated system for physiological measurement and recording (PowerLab/4S, with ETH-400 bridge amplifier; CB Science, Inc.; Dell Optiplex GX1p computer and CHART software; AD Instruments, Hastings, England). The lowest suprathreshold voltage required to produce maximal force and displacement responses of the muscle was determined, and 1.25 times that stimulus voltage was used for the remainder of each individual experiment.

Nerve injury and PEG application in situ

After exposure of the nerve and muscle and arrangement of the stimulation and recording electrodes, baseline values for the force, displacement, and CAPs were established. These data reflect values for the three tests under surgically isolated but uninjured conditions. The sciatic nerve was then crushed for 90 seconds with modified Dumont 5 forceps. The forceps had been filed so that the tip width was 1.5 mm, and they were bent so that the tips were parallel to each other. Preliminary experiments were used to determine the duration of a standard displacement crush required to completely eliminate all three functional measures for a minimum of 30 minutes, allowing only minimal recovery of any one functional response within 60 to 90 minutes after injury (data not shown). Before and immediately after the crush injury, a baseline record of all functional responses to sciatic nerve stimulation was obtained for all animals. Subsequently, 0.05 to 0.1 ml of a PEG or control solution was injected beneath the epineurium, at the site of the crush injury, with a 29-gauge needle on an insulin syringe. A vital dye was included in each solution, to enable direct observation of its removal. Each solution was left in place for 2 minutes. The epineurium was then opened longitudinally with a razor, and the solution was irrigated away with lactated Ringer's solution. Electrophysiological recording was performed at 5-minute intervals for 90 minutes after administration of each solution. The animals were then killed by intracardiac injection of 50 mg of pentobarbital.

The animals that were treated within 10 minutes after injury were divided into the following groups: 1) the PEGtreated group (n = 8), 2) a Krebs' solution-treated control group (n = 6), and 3) a distilled water (vehicle)-treated control group (n = 6). A 50% (by weight) solution of M_r 1800 PEG in distilled water was used for the treated group. Krebs' solution consisted of 124 mmol/L NaCl, 2 mmol/L KCl, 1.2 mmol/L KH₂PO₄, 1.3 mmol/L MgSO₄, 1.2 mmol/L CaCl₂, 10 mmol/L dextrose, 26 mmol/L NaHCO3, and 10 mmol/L sodium ascorbate. We pooled the data obtained for the two control groups (described below) because there was no statistical (or behavioral) difference between them. We do not know of a biologically inert, high-molecular weight surfactant, polymer, or copolymer (e.g., poloxamers, poloxamines, dextrans, or ethylene oxide-propylene oxide-ethylene oxides [EPAN]) that could serve as a control substance. All compounds demonstrated some degree of membrane-sealing and/or fusion ability (see below).

Nerve injury and delayed PEG application

Delayed application of PEG solution or control Krebs' solution was performed 4 hours after crush injury. Six guinea pigs were treated with PEG (50% solution of Mr 1800 PEG), and six animals were treated with the control Krebs' solution. The animals were anesthetized as described previously. Then, a 1-cm segment of the sciatic nerve was exposed at the midhamstring level, and a 90-second crush was performed with the modified Dumont 5 forceps. The wound was irrigated with lactated Ringer's solution and closed with 3-0 silk sutures. The animals were kept under a warming lamp and were reanesthetized 3.5 hours later. The entire sciatic nerve and gastrocnemius muscle were exposed, and the stimulating and recording electrodes and transducers were arranged as described above. Four hours after the crush injury, either PEG solution or Krebs' solution was administered beneath the epineurium, at the injury site, and was removed after 2 minutes by using the techniques described above. Electrophysiological recordings were performed at 5-minute intervals for 60 minutes after administration of each solution. The animals were then killed by intracardiac injection of 50 mg of pentobarbital.

Statistical analyses

Population means were compared with the Mann-Whitney two-tailed test, whereas proportions were compared with Fisher's exact test (two-tailed).

RESULTS

Fusion of severed sciatic nerves in vitro

Our investigation began with the determination of whether any axons within the two segments of severed sciatic nerves could be immediately reunited by PEG application. Four sciatic nerves (approximately 38 mm in length) were tested in this way in the double-sucrose gap chamber. Each was allowed to "recover" from the isolation procedure for varying times up to 1 hour, until the normal capacity to conduct CAPs from the point of stimulation to the recording site on the other end of the chamber had stabilized. The nerve was then completely transected in the middle compartment of the doublesucrose gap chamber, totally eliminating conduction across its length. Earlier trials with spinal cords and pilot trials with severed sciatic nerves demonstrated that CAPs were never observed to arrive at the recording electrodes when the proximal and distal segments were pressed together without PEG or loosely abutted with PEG. Although this may be a foregone conclusion, the test confirmed that there can be no alternate conduction pathway or artifact attributable to the design of the chamber (Fig. 1).

The average CAP magnitudes before the injury were 5, 3.5, 1.5, and 4.8 mV. Stimulation and recording of CAPs were continued during the process of transection, simultaneous with the total elimination of CAP conduction. Within 15 minutes after the mechanical abutting of the proximal and distal segments and a 2-minute application of PEG, CAP conduction was variably restored in all four sciatic nerves tested, with an average recovery of 3.45% of the pretransection amplitude. *Figure 3* presents electrical records for one sciatic nerve tested. In summary, all four attempts to fuse completely severed sciatic nerves were successful, restoring varying levels of



FIGURE 3. PEG-mediated recovery of CAPs in isolated sciatic nerves. The *top trace* shows a CAP stimulated and recorded within the double-sucrose gap chamber. Its complete elimination after transection (*middle trace*) and its partial recovery after abutment of the proximal and distal segments and PEG application (*bottom trace*) should be noted. Although the magnitude of recovery seems small, this reflects hundreds or possibly thousands of individual axons within the sciatic

nerve that have been reconnected and are once again physiologically competent. This recovered CAP was eliminated by retransection (19, 21). nerve impulse conduction through the lesion. Retransection eliminated this recovered CAP propagation across the length of the nerve.

Spontaneous and PEG-mediated recovery of crushed sciatic nerves in situ

Immediately after the crush procedure, the gastrocnemius muscle did not exhibit any response to stimulation of the sciatic nerve in any animal. *Figures 4* and 5 present recordings of all three functional responses before injury to the nerve and demonstrate the complete loss of these responses, as revealed by physiological recordings initiated immediately after the injury. *Figure 4* also demonstrates the lack of response to stimulation 1 hour later in a control animal. This result was typical of those for sham-treated animals. Only 3 of the 12 animals in that group recovered any of the functional responses by 1 hour after treatment.

In contrast, seven of eight PEG-treated animals recovered at least one of the functions within the first 35 minutes after injury. Four of those animals recovered at least one function by 5 minutes after treatment, another by 10 minutes, and two more by 30 minutes. This difference in proportions between control and PEG-treated animals was statistically significant (P = 0.019, Fisher's exact test). This extraordinarily rapid recovery in more than 50% of the animals, in contrast to the complete lack of response in control animals, was observed in pilot trials (data not shown) and in published reports of swift recovery of function after spinal cord injury (4). In the PEGtreated group, one of eight animals recovered all three functional measures by 1 hour after treatment, three of eight recovered two measures, and the balance (four of eight animals) recovered one functional measure, as mentioned above. Figure 5 presents recordings of the three responses before injury and their elimination by the crush injury to the sciatic nerve, similar to Figure 4 but in an animal before treatment with PEG. Figure 6 documents the recovery of two of the three functional measures (CAPs and force of muscle contraction) within the first 5 minutes after PEG application in this animal. The response was robust, necessitating a reduction in the sensitivity of recording. Figure 5 presents data for the only animal (which was PEG-treated) that exhibited all three indices of functional recovery. Overall, the most sensitive and consistent indicator of recovery for all animals was the measurement of muscle contraction force, whereas the least sensitive was the recording of measurable displacement of the hind foot. Furthermore, these responses were infrequent and insufficient for comparison of the mean values of displacement. This latter test was eliminated from the regimen when delayed PEG applications were evaluated (described below). Table 1 provides a summary of these data.

Because of the all-or-none character of muscle excitability, comparison of the mean peak CAP amplitudes would not have been as informative as assessment of the proportion of animals recovering excitability after nerve injury and treatment (*Table 1*). Comparison of the mean force of muscle contraction, however, provided a way to compare the relative degree of muscle "recovery" between the groups. The mean



FIGURE 4. Physiological measurements of gastrocnemius muscle activity in response to stimulation of the sciatic nerve in a sham-treated animal. Electrical recordings of three measures of the response to stimulation are shown in each of the three panels, and the event marker is shown as a *dashed line*. The time scale indicated for the top set of recordings applies to all three sets. The scale units on the *y*-axes are the same for all recordings unless noted. For example, the recording sensitivity is increased by factors of 2 and 10 in the lower recordings of CAPs and force, respectively. It should be noted that the three measurable responses to sciatic nerve stimulation were completely eliminated immediately after a crush injury of the nerve proximal to its insertion in the muscle. It should also be noted that this lack of response was stable for the next 1 hour, even with increased sensitivity of recording.



FIGURE 5. Physiological responses of the gastrocnemius muscle to stimulation of the sciatic nerve in a PEG-treated animal. The conventions are the same as detailed for *Figure 4*. The 10- and 100-fold increases in recording sensitivity for CAPs and force immediately after a crush injury to the sciatic nerve and the inability to measure any response at that time, with that sensitivity, should be noted. The rapid robust recovery of CAPs and muscle contractile force, as emphasized by the substantial reduction in amplifier gain required to record those responses at the 5-minute time point, should also be noted. This recovery was stable for the next 1 hour of monitoring.





contraction force was significantly improved by PEG treatment (mean, 23,835 \pm 19,991 dyn; range, 0–163,000 dyn), compared with control values (mean, 433 \pm 276.3 dyn; range, 0–2880 dyn; *P* = 0.008, Mann-Whitney two-tailed test).

We did not detect any difference (behavioral or statistical) between the groups when a 1-hour-delayed application of PEG was tested (data not shown). A comparison of shamtreated and PEG-treated animals after a 4-hour-delayed ap-

	No. of Animals	No. with Measurable Function before Injury	No. with Measurable Function after Injury	Elapsed Time after Treatment (No./Total No. Tested) ^b				<i>P</i> Value ^{<i>c</i>}
				5 min	15 min	30 min	60 min	
Control	12	12/12	0/12	1/12		1/12	1/12	0.019
PEG-treated	8	8/8	0/8	4/8	1/8	2/12 ^d		

TABLE 1. Responses to Immediate Application of Polyethylene Glycol^a

^a PEG, polyethylene glycol.

^b Elapsed time to recovery after application.

^c Statistical comparison of totals (Fisher's exact test, two-tailed).

^d One of these preparations recovered at approximately 35 minutes.

plication was informative, however, revealing the ability of PEG to still improve functional outcomes. The proportion of control animals (two of six animals) that demonstrated recovery of at least one functional measure 4 hours after injury did not change after the sham application. After PEG application, however, this number quadrupled (one of six animals demonstrated spontaneous recovery by the time of the 4-h application, which improved to four of six animals within 5–45 min after treatment). Because of the small number of animals, this increase in proportion, relative to control animals, did not reach significance but exhibited a strong trend in that direction (P = 0.06, Fisher's exact test).

DISCUSSION

Using both in vitro and in vivo assessments of physiological functioning in cut or crushed sciatic nerves, we established that a brief (approximately 2-min) topical application of PEG was sufficient to restore variable levels of electrophysiological conduction through the lesion. This was demonstrated by PEG-mediated reconnection of completely severed sciatic nerves maintained within a double-sucrose gap recording chamber and by in vivo evaluation of muscle excitability after a severe proximal crush injury to the motor efferent. In the latter assays, we simultaneously monitored two other indices of muscle responsiveness, i.e., the force of gastrocnemius muscle contraction and the displacement of the muscle after electrical stimulation of the sciatic nerve. As described, muscle displacement was the most crude and least sensitive measure of functional recovery. Measurement of recovering muscle CAPs was of intermediate successfulness. Sometimes CAPs traversing the muscle preparation could not be measured, although simultaneous gastrocnemius muscle contraction was visible and measurable with the force transducer. This was not completely unexpected, given the difficulty of measuring extracellular CAPs in a blood field.

With both testing procedures, PEG application resulted in rapid (minutes) recovery of functioning, at a time when control preparations had not spontaneously recovered. In in vitro transection experiments, recovery of CAP propagation in the absence of a stable reconnection was not observed (25). In in vivo experiments, recoveries were so rapid (<1 h) for most treated animals that blinding procedures were not required (4). Simply stated, the extremely rapid reversal of deficits mediated by PEG could not be confused with the much slower mechanisms of endogenous repair (see discussion in Reference 23). This was easiest to detect when PEG was applied within minutes after the injury, although a 4-hourdelayed application of PEG greatly increased the percentage of animals that recovered at least one functional measure. The smaller number of animals studied at these later times did not allow significance to be reached, although a strong trend toward significance was noted. We did not attempt survival surgery experiments in these studies, because of the considerable dissection of the branches of the sciatic nerve for isolation of its projection to the gastrocnemius muscle. Determination of the persistence of PEG-mediated recoveries and separation of these recoveries from recovery based on regeneration of proximal segments are not trivial and require an alternate injury model and means of evaluation. Such studies are ongoing.

In in vivo spinal cord injury experiments, the recovery of physiological or behavioral functions induced by PEG was stable for 1 month of observation (4). Moreover, these functions continued to improve with time for all animals, whereas spontaneous recovery among control animals was worse with time for some animals (20).

Mechanical damage to axons and its reversal by PEG

It is now clear that, in response to mechanical damage to nerve membranes, the instantaneous primary insult is local breakdown of the ability of the membrane to act as an ionic fence. Electrophysiological conduction is impaired or eliminated by sustained collapse of the membrane potential at the site of damage, which is caused by the unregulated exchange of ions (principally K⁺ and Na⁺). The anatomic integrity of axons is compromised by the local increase in the levels of Ca^{2+} entering the cytosol at the foci of damage (3, 15). This increase in intracellular Ca²⁺ levels resulting from unregulated entry into the cell is exacerbated by increasing intracellular Na⁺ levels, which initiate the release of Ca²⁺ from intracellular stores (3, 7, 14, 21). The net result is depolymerization of the cytoskeleton, activation of various intracellular, Ca²⁺-dependent, catabolic enzymes, and other biochemical processes, leading to local progressive cellular dissolution, axotomy in the most extreme cases, and degeneration of distal axonal segments in mammals. The duration of this process is variable, depending on many characteristics of the damaged nervous tissue and the severity of the impact, compression, or stretch; however, the process may require many hours to days. It is our working hypothesis that this process can be greatly reduced in scope with the acute use of hydrophilic polymers, which can seal even the worst breaches of axonal membranes. This effect can be demonstrated by the ability of PEG to actually fuse proximal and distal segments of axons, restoring anatomic and physiological functioning (25). In less severe injuries to the axolemma, the action of the polymer likely does not enhance the natural reparative mechanism of sealing by axons, because this process requires a much longer time than does sealing mediated by PEG (23, 24). Natural or spontaneous sealing of crush injuries to the sciatic nerve and resultant functional recovery were demonstrated in this study. This recovery was enhanced by PEG application. In the most severe cases of mechanical damage to axons, PEGmediated sealing likely prevents axotomy, immediately permitting CAP conduction through the injury site and producing variable levels of immediate functional recovery dependent on nerve impulse conduction. Below, we detail the experimental evidence supporting these hypotheses.

Actions of PEG on damaged membranes

There are several hypotheses regarding the ability of large hydrophilic polymers, such as PEG and related compounds (the triblock polymers), or nonionic detergents to reverse cell permeabilization. In certain cases and at lower molecular weights, PEG may have detergent-like properties similar to those of amphiphatic polymers (poloxamers and poloxamines). These compounds may form thin micellar films, covering the breach in the membrane. In the case of the triblock polymers, the hydrophobic "head" of the molecules may actually insert itself into the breach in the membrane, because the hydrophobic core of the plasmalemma is exposed by the injury. The hydrophilic PEG "tails" are integrated with the outer leaflet. It is also possible that PEG seals porated membranes via acute dehydration of the local region where it is applied. This is envisioned to enable the structural elements of the membrane (e.g., proteins and glycolipids) to resolve into each other, because the polar forces arising from the aqueous phase (helping to maintain the organization within the membrane) are absent or reduced. When PEG is removed and the local membranes are rehydrated, spontaneous reassembly of these structural elements leads to restoration of the membrane. This hypothesis helps explain the immediate recovery of excitability after topical PEG treatment of nerve membranes and the success of only brief applications of the polymer to injured tissues. We and others have discussed these putative mechanisms of action underlying the polymermediated fusion and repair of traumatized cell membranes (4, 10, 13, 16, 23).

In general, the use of these families of high-molecular weight polymers represents a new approach, as well as a practical means, for the treatment of acute trauma to tissues caused by the primary breakdown of cell membranes. This includes a reversal of cell permeabilization in different injury models, including electric shock myonecrosis (11), testicular reperfusion injury (20), heat shock-mediated cell death (19), and radiological damage to cells (8).

Repair of nervous system damage with PEG

We have been testing the ability of the hydrophilic polymer PEG to fuse and seal the axolemma after mechanical damage. This approach differs from all other experimental and clinical repair strategies, most of which are designed to facilitate endogenous regeneration of peripheral nerve proximal segments and to provide more-beneficial fasciculation pathways for regenerating axons, through the use of tubulization and/or additional substrates and substances, such as growth factors. These other techniques, as applied to the peripheral nervous system, are basically regeneration strategies, whereas PEG administration provides a means to rescue cells from secondary axotomy or possibly to reconnect their severed axonal segments.

Our studies first used ventral spinal cord white matter isolated from adult guinea pigs. Spinal cord strips were maintained in the double-sucrose gap recording chamber and were completely transected and then fused with PEG (25) or crushed within the chamber and then repaired with PEG (23). In both cases, a 2-minute topical application of the polymer (approximately M_r 1400–1800) rapidly restored physiological conduction of CAPs through the lesion, within minutes after the injury. Moreover, intracellular labeling with two different fluorescent markers (rhodamine and fluorescein-labeled dextrans) demonstrated that physiological functioning after spinal cord transection and PEG treatment was accompanied by restored anatomic integrity of axons across the transection plane, via the physical reattachment of their proximal and distal segments (25).

We further tested the ability of PEG to repair a severe standardized compression injury of the spinal cord in vivo. We performed extracellular stimulation of the tibial nerve of the hind leg and recorded volleys of evoked potentials arriving at the contralateral sensorimotor cortex (so-called somatosensory evoked potentials) as an index of electrophysiological recovery, and we monitored the recovery of the cutaneus trunci muscle reflex as a index of behavioral recovery after severe spinal cord injury. Spontaneous recovery from spinal cord injury via natural mechanisms of repair occurs in less than 20% of the animals monitored for a minimum of 1 month after injury. A 2-minute PEG application to the exposed spinal cord injury immediately after the crush (or delayed for 7–8 h) resulted in recovery of the cutaneus trunci muscle reflex in more than 90% of the treated population. A standardized lesioning technique (17) also resulted in complete loss of somatosensory evoked potential conduction in the spinal cord in 100% of the injured animals, and there was no recovery of conduction through the lesion with any of the control treatments. A striking and unexpected result was the recovery of somatosensory evoked potential conduction in 100% of PEGtreated animals, usually a few hours to 1 day after treatment (4). By using a dye exclusion test, we have also determined that this brief PEG application indeed seals spinal cord axons (24). Brief exposure of acutely injured isolated guinea pig white matter to a solution of horseradish peroxidase led to endocytosis of the label, marking only damaged axons. Most axons in the crushed spinal cord took up the label. A striking and statistically significant reduction in horseradish peroxidase uptake was associated with a 2-minute PEG treatment before horseradish peroxidase exposure, demonstrating that the polymer sealed these breaches (24).

Clinical relevance

This ability of PEG to functionally reconnect severed axons is the most challenging test of its reparative capability. The organization of the nerve trunk into fascicles and the tough surrounding epineurium of the sciatic nerve raised the possibility that fusion of even some of the axons inside might not be possible, particularly because of the possibility of their retrograde degeneration away from the plane of transection within the fascicles (1). We learned that the sciatic nerve was similar to spinal cord white matter, in that each attempt to fuse some axons within the cut nerve was successful. For spinal cord, this has only limited importance for clinical injuries, because spinal cord transection is rare. However, this is a meaningful result in the context of neurosurgical reattachment of severely damaged peripheral nerves, where the ends of damaged nerve trunks may be resected before fascicular alignment and suturing. Additional treatment with PEG requires procedures to stabilize the perhaps delicate fused regions for an undetermined period of time. This additional therapy may yield dividends in permitting greater immediate functional recovery while eliminating variable degrees of Wallerian degeneration and muscle atrophy. We decided to begin such explorations with the nonsurvival procedures reported here, because long-term (days to weeks) monitoring of animals with peripheral nerve injuries is complicated. Comparisons of functional test results are difficult because of the robust spontaneous repair and regeneration of rodent peripheral nerves. These data have convinced us that the next steps in our investigations should be careful evaluation of the longterm functional repair of peripheral nerves by PEG and definition of the critical time window in which application is required. With respect to spinal cord repair using PEG, we have already moved these techniques into clinical testing, using naturally occurring cases of neurologically complete paraplegia in dogs (2, 5, 6).

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COMMENTS

Polyethylene glycol (PEG) was compared with water or Krebs' solution for the treatment of acutely severed guinea pig sciatic nerves suspended in a double-sucrose gap chamber. An in vivo preparation involving crushed sciatic nerve was used for comparisons of compound action potentials (CAPs), muscle contraction force, and millimeters of muscle displacement in one contraction.

Very small CAPs (3.56% of normal values) were restored by 1 hour after axonal transection in PEG-treated nerves, presumably because of some resealing of axonal membranes. In the in vivo experiments, PEG-treated animals exhibited some recovery of contraction force and muscle displacement, although CAPs could not be measured.

The authors conclude that PEG application may be a means to interfere with what they term the "steady dissolution of peripheral nerve fibers," which is better know as the Wallerian degenerative process, after mechanical damage. Such observations could have potentially immense importance, especially if PEG could be applied after a delay of hours to days after injury, which would be necessary for its use to have any practical application. Most human nerve injuries are not as focal or neat as those in this experimental model. Instead, they involve relatively long lengths of nerves and much larger forces, and thus much more extensive membrane injuries, than studied here. Sharply transected nerves are much less frequently observed than are contused or stretch-injured nerves. In this regard, the crush injury used in these experiments was relatively mild, because even in the control nerves some return of function was evident within 1 hour for 3 of 12 animals. Human crush or contusive injuries are much more destructive and would be expected to be less amenable to nonmechanical corrections. Nevertheless, the observations in this article are important and suggest that such metabolic interventions may hold some promise in the future. It would be interesting to assess, in an in vivo severance model, shamversus PEG-treated animals and to determine whether, in a period of weeks to several months, PEG treatment could prevent the Wallerian process via relatively early sealing together of the axonal membranes.

From a neurophysiological perspective, if the stimulation in the divided-axon model is strong enough, it can jump to the distal axon, which can conduct for up to 72 degrees after a more proximal division. Similarly, muscle remains active for a number of days after axonal crush or division. Once again, if the stimulus is applied close to the injury site or with enough amperage to traverse the injury site, then acutely injured muscle can be stimulated to contract. This would be a very important experiment; if PEG does not produce such effects with acute, low-energy, sharp or mild crush lesions in guinea pigs, then a different route must be explored.

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This basic research article reports on the application of PEG to peripheral axons. The authors used an in vitro preparation in which guinea pig sciatic nerves were transected and an in vivo preparation in which similar nerves were crushed. They convincingly demonstrate a modest but significant recovery of CAP amplitude after in vitro application. In the more clinically relevant paradigm, subepineurial injection of PEG to crushed sciatic nerves seemed to increase the proportion of nerves (relative to placebo-treated control animals) that demonstrated recovery of CAPs and contractions measured in the gastrocnemius muscle. It was speculated, but not demonstrated, that there may be electrophysiological recovery, dissolution of axons after injury, and/or fusion of the proximal and distal axonal stumps.

The authors pursued a relatively novel approach to peripheral nerve repair, in that their strategy represents an attempt to either prevent or immediately repair axonal damage resulting from the initial insult. This strategy differs from much basic science research on nerve injuries, which focuses on strategies to enhance nerve regeneration.

The findings by the authors are intriguing but very preliminary. The enhanced physiological recovery was observed only after acute application, whereas delayed application (1 or 4 h after the crush injury) was associated with either no or nonsignificant differences in outcomes, compared with control animals. An insufficient sample size may not have allowed statistically significant differences to be determined. A detailed analysis of the kinetics of application and its effects would be worthwhile for the authors to pursue. Specifically, is PEG effective only if it is immediately applied or can delayed application 8, 12, or up to 24 hours after injury still have a positive effect? This is critically important, because many clinically encountered nerve injuries are observed several hours or more after trauma.

Although physiological demonstration of conduction suggests axonal repair, more-direct anatomic evidence of axonal persistence is needed. Perhaps the approach of using anatomic tracers such as horseradish peroxidase should be pursued in this model, as the authors have done in spinal cord studies. Specifically, the demonstration of persistent axons, a few days after axotomy, after cut (not crushed) sciatic nerve injuries would be much more convincing evidence. Finally, longer-term studies evaluating functional recovery would

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substantiate the physiological findings reported here. We look forward to further findings from this laboratory regarding the promising role of PEG in the repair of peripheral nerve injuries.

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This study supports the view that PEG application may enhance the functional recovery of damaged axons in guinea pig models involving completely severed sciatic nerves and standardized crush injuries. Although the empirical evidence and observations reported are quite convincing, it is still difficult to determine, on the basis of this study and similar studies, to what extent the results obtained with these models are translatable to clinical situations in human patients and/or primates, where more complex organization of the nervous system may produce somewhat higher expectations regarding functional recovery. It is fascinating that PEG may act as at least a temporary glue in these cases, but the exact mechanism for these actions and whether the effects observed with PEG are specific or nonspecific remain unclear. Nevertheless, more mechanistically oriented studies should provide at least partial answers to these extremely important questions.

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