Use of a static magnetic field to promote recovery after peripheral nerve injury

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Object. While pulsed electromagnetic stimulation has been shown to enhance peripheral nerve regeneration, the effect of a static magnetic field on nerve repair is less clear. The aim of this study was to establish what effect an imposed exogenous static magnetic field has on peripheral nerve regeneration after transection and repair.

Methods. Three groups of six adult sheep were used. The first group acted as normal controls. In the second group, the median nerve was divided and immediately repaired by entubulation within a “controlled-release” biodegradable glass tube. In the third group, small magnets were applied to the sides of the biodegradable glass tubes before the median nerve was repaired using these magnetic tubes. The sheep were allowed to recover and were reexamined 10 months later. The animals underwent comprehensive morphometric (cross-sectional morphometry and measurement of internodal lengths), electrophysiological (determinations of stimulated jitter, maximum conduction velocity, refractory period, and F waves), and isometric tension (isometric twitch and tetanic tension) assessments.

Conclusions. Exogenously applied static electromagnetic fields do not enhance peripheral nerve regeneration.

Key Words • electromagnetic field • nerve regeneration • sheep

Abbreviations used in this paper: CRG = controlled-release glass; EMG = electromyography; FCR = flexor carpi radialis.
Static magnetic field and peripheral nerve regeneration

to assess what effect an imposed exogenous static magnetic field has on peripheral nerve regeneration after transection and repair.

Materials and Methods

Description of Groups

All experiments were performed in accordance with guidelines set forth in the United Kingdom Animals Scientific Act, 1986. Three groups of six adult sheep were used. The first group acted as normal controls. In the second group the median nerve was divided and immediately repaired by entubulation within a biodegradable glass tube. In the third group small magnets were applied to the sides of the biodegradable glass tube before the median nerve was repaired by using the now magnetic tube. These animals were allowed to recover and were reexamined 10 months later.

“Controlled-Release” Glass Tubes

Biodegradable “controlled-release” inorganic polymer glass tubes can be manufactured to fit the dimensions of any nerve, and their rate of solubility can be adjusted to encompass the time required for nerve regeneration. They have been used in a number of biological applications. In these experiments CRG tubes were used as the frame on which we mounted magnets.

Application of Magnets

Circumferential magnets were applied to the outside of the CRG tube. The field strengths varied along the length of the tube to approximately 30 milliteslas. The magnets were oriented with the cathode end placed distal to the site of repair (Fig. 1).

Anesthesia Induction and Maintenance

Anesthesia was induced by giving each sheep a bolus dose of intravenous thiopentone. Anesthesia was maintained by administering a mixture of oxygen, nitrous oxide, and vaporized halothane. All animals undergoing recovery experiments were infused with 1 L normal saline perioperatively. A prophylactic antibiotic, cefuroxime, was given intramuscularly at the start of the operation.

Surgical Management

Under sterile conditions, an incision was made proximally in the lateral chest wall and extended distally to the groove formed between the FCR muscle and radius, distally. The median nerve was dissected out in the axilla and forearm. A 4- to 5-cm portion of the nerve was dissected off the brachial artery, and the nerve was then transected using a Meyer neurotome. The biodegradable glass tube was threaded onto the proximal end of the median nerve. A tension-free repair was performed using four or five simple epineurial sutures with the aid of an operating microscope. The CRG tube was then slid distally along the nerve so that its center was positioned over the repair site. We anchored the glass tube by passing a 5-0 polyamide suture through 1-mm holes at the ends of the tube, and secured the tube to the underlying muscle and fascia.

Sheep in the third group underwent a procedure that was similar to that used in the CRG tube group, except that a circumferential magnet was applied to the tube prior to entubulation. A Sapphire EMG machine (Medelec, Old Woking, United Kingdom) was used for all stimulating and recording purposes. In the first test jitter was recorded. The measurement of jitter involves stimulating the terminal motor fibers of a nerve several times and then recording the variability in the muscle-fiber action potential for each stimulation. The equations used to calculate jitter and a detailed method of the procedures are provided in previous papers. The techniques described here constitute a brief summary of that method.

The muscle chosen for study was the FCR because it is supplied solely by the median nerve. The terminal branches of the median nerve were stimulated using two monopolar needle electrodes (MF-37; Medelec). One needle electrode was inserted into the motor portion of the FCR muscle and was used as the stimulating cathode. The second needle electrode was inserted approximately 1 cm proximal and 1 cm lateral to the cathode and used as the anode. A square-wave current with a duration of 50 μsec and an amplitude of 1 mA was applied between these electrodes to stimulate a terminal nerve fiber. The stimulus frequency was 1 Hz. The current was increased by 0.1 mA increments until small twitches were observed in the FCR muscle. At this point a single-fiber EMG needle recording electrode was inserted into the twitching portion of the muscle belly, approximately 2 cm distal to the cathode. The current amplitude was increased, and the single-fiber EMG needle was adjusted until it was possible to record a repeatable muscle-fiber action potential. Fifty muscle-fiber action potentials were recorded, and the mean consecutive difference was calculated. This process was repeated for 20 different motor units. The average of the 20 mean consecutive differences was called the jitter for that muscle.

After the recording of the jitter test, the wound was reopened and the median nerve dissected out. Two nontraumatic low-impedance platinum-wire stimulating electrodes were placed under the median nerve. The distal electrode was placed where the nerve emerges from beneath the superficial pectoral muscle. The proximal electrode was situated immediately distal to the brachial plexus. The cathode was positioned so that it was distal to the anode. Two 6-mm silver/silver chloride disc recording electrodes were placed on the skin over the FCR muscle to record the electrical activity of the muscle during contraction. The cathode was placed on the muscle belly with the anode near the insertion of its tendon. A ground electrode was placed on the skin over the sternum.

Fig. 1. An illustration showing the “magnetic (magnet) tube” encasing the nerve repair site.
The following electrophysiological indices were measured: 1) maximum conduction velocity; 2) refractory period; and 3) the minimum latency of the F wave. The maximum motor conduction velocity was calculated from the difference in latencies of the compound muscle action potentials (M waves) when the nerve was stimulated at the proximal and distal sites, divided by the distance between the two sites.

The absolute refractory period was recorded using a paired-shock technique. A supramaximal stimulation was applied to the median connector for the maximum nerve from the distal stimulating electrode, and the resulting M wave was recorded as a reference trace. Increasing time delays were introduced until such time that a change was detected in the M wave. This change represented the start of a second M wave. In practice, the second M wave was detected by digitally subtracting the reference trace from the active trace. The time delay at which the second M wave appeared was called the absolute refractory period.

An F wave is a compound action potential evoked intermittently from a muscle by a supramaximal electric stimulus to a nerve. These F waves result from backfiring of antidromically activated anterior horn cells. An optimal display of F waves required a sensitivity of 100 to 500 mV, a sweep speed of 10 to 20 mm/second, and a stimulation rate of 2 pulses/second. Repetitive supramaximal stimulations were applied to the nerve until 20 traces were stored. Each trace was then inspected separately for the presence of an F wave.

**Isometric Tension**

Once the electrophysiological tests had been completed, the tendon of the FCR muscle was dissected out and attached to a 200-N force transducer (PFI 200N; Memesin, West Sussex, United Kingdom) by using an inextensible suture. The signal from the force transducer was amplified before it was attached to the Y connector of an oscilloscope. The RS-232 output from the EMG machine was connected to the trigger input of the oscilloscope, allowing the EMG machine to trigger the sweep of the oscilloscope when stimulation of the nerve was initiated. Isometric twitch and tetanic tensions were then measured before the muscle was dissected out and weighed.

**Morphometric Analysis**

At the end of the experiment two sections of the median nerve distal to the repair site were harvested and placed in fixative solutions. Following this, the animal was killed by an overdose of pentobarbital sodium. The first section of the nerve was washed with sucrose buffer and placed in a solution containing osmium tetroxide. Osmication is essential for myelin preservation. After 3 hours the osmium was removed, and the specimen was dehydrated in a series of graded alcohols. The nerves were cut into semithin (1-μm) sections before they were stained with toluidine blue. The nerve sections were examined with the aid of a compound microscope (Zeiss, Oberkochen, Germany) at a magnification of 400. The morphometric analysis, which included measurements of axon diameter, fiber diameter, and myelin sheath thickness, was performed using digitized images of the sections and aided by a computerized image analysis system (version 3.0, Analytical Imaging System; Isee Imaging Systems, Raleigh, NC), which had been calibrated using a standard 1-mm graticule. The axon and fiber diameters of 200 nerve fibers were measured from each section by using a random-sampling technique.

The other section of nerve was stripped of its epineurium, and the individual fibers were teased apart. After fixation in formalin, the specimen was osmicated for 3 hours. With the aid of a dissecting microscope, we slowly and carefully teased apart the fascicles. One or two fascicles were placed on a slide, and further dissection was then needed to unravel the individual fibers. Twenty randomly chosen internodal segments were measured on digitized images of the sections by using a computerized image analysis system (Analytical Imaging System) that had been calibrated.

**Statistical Analysis**

Raw data were first organized in a computer spreadsheet (Excel; Microsoft Corp., Redmond, WA). The statistical program Statistica (StatSoft, Tulsa, OK) was used for all statistical analyses and for creating graph plots. Half-normal plots were constructed to help identify outliers and reject outliers from within the raw data. After rejecting the outliers the columns of data were replotted as normal probability plots to determine whether the data fit a normal distribution.

The next two stages in the statistical testing were directed at identifying the presence of differences (variants of the F test) and identifying, by means of post hoc tests, where those differences lay (variants of the Student t-test). Different algorithms had to be adopted for normally and nonnormally distributed data. For normally distributed data, the F test was applied in the form of a one-way analysis of variance. To find where these differences lay the Scheffé test was performed.

For data that were not parametrically (normally) distributed the Kruskal–Wallis test was used. For the post hoc tests we used both the Mann–Whitney U-test (physiological and tension data) and the Kolmogorov–Smirnov test (morphometric data).

**Results**

Prior to undertaking these experiments, we calculated the required sample size by using the Kirkwood formula. According to the work of Fullarton, et al., for the maximum motor conduction velocity, if we expect an appreciable effect to be a difference of 15 m/second between groups with a standard deviation of 7 m/second, the calculated group size should be 4.59. Application of this principle to all the variables measured experimentally indicated that a minimum group size of five sheep was acceptable. No early or late postoperative complications were detected in any of the animals. When the sheep were reexamined, it was found that, following nerve repair, animals in both the CRG tube and magnetic tube groups had a reduction in all electrophysiological, isometric tension, and morphometric variables when compared with control animals.

There was no apparent statistical difference between the CRG tube and magnetic tube groups for conduction velocity (Fig. 2). Differences between the CRG tube and magnetic tube groups were apparent for myelin sheath thickness according to the morphometric assessment (Fig. 3), but no statistical difference was found in axon diameter, fiber diameter, or internodal length (Fig. 4).

Muscle mass and isometric tetanic tension were reduced in animals in all groups after nerve repair. The percentage of...
preserved muscle mass was significantly greater in the CRG tube group (68% in the CRG tube group and 52% in the magnetic tube group, \( p = 0.01 \); Fig. 5). The percentage of tetanus preserved after nerve repair was greater in the CRG tube group (53% in the CRG tube group and 41% in the magnetic tube group; Fig. 6); however, this difference was not statistically significant. No difference was found in any of the other morphometric, electrophysiological, or tension tests that were performed. Power analysis studies were conducted for each of the dependent variables and produced a value greater than 0.8 in all cases.

**Discussion**

The use of pulsed electromagnetic fields has been shown in the experimental and clinical literature to promote healing of nonunions in bone.\(^1\)-\(^3\) Papatheofanis and associates\(^2\) demonstrated that pulsed electromagnetic fields were not necessary for the induction of skeletogenesis and, instead, that intense static magnetic fields may induce bone growth in vivo. Cordeiro, et al.,\(^1\) assessed what effect a high-intensity magnetic field would have on peripheral nerve regeneration. They transected and repaired sciatic nerves in rats. These experimental animals were then restrained or not and oriented north or south in a 1-tesla (10,000-gauss) static magnetic energy field 12 hours per day for 4 weeks. The experimental animals and those in a control group were studied by obtaining electrophysiological recordings and quantitative axon counts. No differences were found in the compound action potentials or myelinated axon counts. Origel and colleagues\(^2\) highlighted several flaws in this work. First, the source of the magnetic field was chosen because of its availability (presence of a magnetic resonance imager). However, this field had 500 to 1000 times the energy...

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**Fig. 3.** Composite box-and-whisker plots (box, mean ± SEM; and whisker, SD) of axon diameter (squares), fiber diameter (circles), and myelin sheath thickness (triangles) for the normal control, CRG tube, and magnetic tube groups.

**Fig. 4.** Box-and-whisker plots (box, mean ± SEM; and whisker, SD) of internodal lengths in the normal control, CRG tube, and magnetic tube groups.

**Fig. 5.** Box-and-whisker plots (box, mean ± SEM; and whisker, SD) of the percentages of muscle mass preserved after repair in the CRG and magnetic tube groups.

**Fig. 6.** Box-and-whisker plots (box, mean ± SEM; and whisker, SD) of the percentages of isometric tetanic tension preserved after repair in the CRG and magnetic tube groups.
of pulsed electromagnetic fields that have proved successful in effecting neural regeneration. Second, the period of magnetic field treatment was arbitrarily chosen. Four weeks was quite early and the regeneration cycle would not have been complete. Third, myelinated axon counts used as an isolated histological criterion of regeneration was not sufficient. Fourth, no attempt was made to quantify function.

The results of the present experiments provided a comprehensive evaluation of the effects of a static magnetic field on peripheral nerve regeneration. The regenerating nerves were exposed to field strengths similar to those used in pulsed electromagnetic experiments that have proved to be beneficial for nerve regeneration. The animals were reexamined after 10 months, allowing sufficient time for completion of nerve regeneration in the animal model used. Comprehensive electrophysiological, isometric tension, and morphometric studies were conducted. There was increased myelin sheath thickness found in the CRG tube group compared with the magnetic tube group. The CRG tube group was also found to have an increased percentage of tetanus and muscle mass preserved after nerve repair. Given that the CRG tubes were used to enslave the repair site in both groups, one is allowed to deduce that the magnetic field was responsible for the poorer regeneration seen in animals in the magnetic tube group. The negative findings seen in the magnetic tube group, although disappointing, make an emphatic statement. Taking these results together with those of Cordeiro, et al., one can say conclusively that exogenously applied static electromagnetic fields do not enhance peripheral nerve regeneration. Indeed the static magnetic field appears to have a detrimental effect on the regeneration process, as illustrated in the measurements of myelin sheath thickness and muscle mass. Although the cathode end was aligned distally to encourage growth across the injured stumps, it may have been that the anode end, which was placed more proximally, was repulsive to regrowing fibers.

Conclusions

Exogenously applied static electromagnetic fields do not enhance peripheral nerve regeneration. The proper and minimum intensity of the electromagnetic field required for nerve regeneration have yet to be determined. The most important factor does not seem to be the intensity of the electromagnetic field, but rather the fact that it is pulsed.

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References

Static magnetic field and peripheral nerve regeneration


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