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Original research

Pulsed electromagnetic fields accelerate functional recovery of transected sciatic nerve bridged by chitosan conduit: An animal model study

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HIGHLIGHTS

- Effect of body exposure to pulsed electromagnetic fields on transected nerve regeneration was assessed.
- Body exposure to PEMF improved functional recovery and morphometric indices of sciatic nerve.
- PEMF combined with chitosan grafting could be an effective, safe and tolerable treatment in clinical practice.

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ABSTRACT

Introduction: Effect of whole body exposure to pulsed electromagnetic fields (PEMF) on nerve regeneration in a rat sciatic nerve transection model was assessed. **Methods**: Sixty male white Wistar rats were divided into four experimental groups ($n = 15$), randomly: In transected group (TC) left sciatic nerve was transected and stumps were fixed in adjacent muscle. In chitosan group (CHIT) the defect was bridged using a chitosan conduit filled with phosphate-buffered saline. In treatment group (CHIT/PEMF) the whole body was exposed to PEMF (0.3 mT, 2 Hz) for 4 h/day within 1–5 days. In normal control group (NC) sciatic nerve was only dissected and manipulated. Each group was subdivided into three subgroups of five animals each and nerve fibers were studied 4, 8 and 12 weeks after surgery. **Results**: Behavioral, functional, electrophysiological, biomechanical, gastrocnemius muscle mass findings and morphometric indices confirmed faster recovery of regenerated axons in CHIT/PEMF than in CHIT group ($p < 0.05$). Immunohistochemical reactions to S-100 in CHIT/PEMF were more positive than that in CHIT group. **Discussion**: Whole body exposure to PEMF improved functional recovery and morphometric indices of sciatic nerve. Detailed mechanism of neuroprotective action remains to be investigated. **Conclusion**: PEMF combine with chitosan grafting could be considered as an effective, safe and tolerable treatment for peripheral nerve repair in clinical practice.

1. Introduction

Pronounced improvements in the diagnosis and repair of transected peripheral nerves are results of technological advances in diagnostic imaging, neurosurgical instrumentation and the use of a surgical microscope [1]. The ideal surgical repair technique should accomplish good wound healing with minimal scar formation and direct the nerve sprouts into their correct targets [2]. The conduits act to guide axons sprouting from the regenerating nerve end, provide a microenvironment for diffusion of neurotrophic and neurotropic factors secreted by the injured nerve stump, as well as help protect from infiltration of fibrous tissue [3].

Different graft equivalents have also been applied to bridge the nerve stump and regulated through the interaction of a variety of protein and cell signals [4]. Biodegradable nerve guides as a temporary scaffold are better than non-degradable biomaterials because the latter remain in situ as a foreign body and ultimately result in limiting recovery of nerve function [5]. Nevertheless, the

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resistance to biodegradation can be a cause of chronic nerve compression in the long run and a second surgery may therefore be required for its removal. Beneficial effects of chitosan as a conduit in promoting nerve regeneration have already been documented and it seems chitosan as a natural polymer has excellent properties including biocompatibility, biodegradability, non-toxicity and adsorption properties, and might be a suitable functional material for peripheral nerve regeneration [6–8].

Pulsed electromagnetic fields (PEMF) are reported to promote peripheral nerve regeneration to an extent similar to that observed with conditioning lesions, growth factors, and hormones [9]. Exposure to PEMF as a pretreatment prior to crush injury has resulted in acceleration of axonal regrowth, and consistent with the stimulation of regenerative neurite outgrowth increased functional outcomes such as walking behavior [10–13]. PEMF has also been shown to promote neurite outgrowth *in vitro* [14].

Others have demonstrated that prolonged PEMF regimen had led to delayed histological peripheral nerve regeneration and increased oxidative stress but no loss of function recovery [15].

These contradictory results were probably due to technical differences, specifically to different protocols for PEMF exposure. Therefore, the present investigators concluded that the issue was not clear and that more experiments were needed to assess the possible benefits of PEMF exposure on peripheral nerve regeneration.

Furthermore, Promising results regarding the beneficial effect of PEMF on transected peripheral nerve regeneration are poor and not supported by functional tests, to the best of knowledge of the authors, which play a crucial role in the assessment of functional nerve recovery.

The present study was conducted to study functional effects of PEMF on peripheral nerve regeneration. Assessment of the nerve regeneration was based on behavioral, functional, electrophysiological, biomechanical, histomorphometric and immunohistochemical (Schwann cell detection by S-100 expression) criteria 4, 8 and 12 weeks after surgery.

2. Materials and methods

2.1. Study design and animals

Sixty male white Wistar rats weighing approximately 290 g were divided into four experimental groups ($n = 15$), randomly: In transected group (TC) left sciatic nerve was transected and stumps were fixed in adjacent muscle. In chitosan group (CHIT) the defect was bridged using a chitosan conduit filled with phosphate-buffered saline. In treatment group (CHIT/PEMF) the whole body was exposed to PEMF (0.3 mT, 2 Hz) for 4 h/day within 1–5 days. In normal control group (NC) sciatic nerve was only exposed and manipulated. Each group was further subdivided into three subgroups of five animals each and surveyed 4, 8 and 12 weeks after surgery. Two weeks before and during the experiments, the animals were housed in individual plastic cages with an ambient temperature of (23 ± 3) °C, stable air humidity and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. All measurements were made by two blinded observers unaware of the analyzed groups.

2.2. Preparation of chitosan conduit

Chitosan solution was prepared dissolving medium molecular weight, crab shell chitosan (~400 kDa, 85% deacetylated) (Fluka, Sigma–Aldrich St. Louis, MO, USA) in an aqueous solution (1% v/v) of glacial acetic acid (Merck, Darmstadt, Germany) to a concentration of 2% (w/v) while stirring on a magnetic stirrer-hot plate. The solution was stirred with low heat (at 50 °C) for 3 h. The resultant chitosan solution was filtered through a Whatman No. 3 filter paper then vacuum filtration to remove any un-dissolved particles. To overcome the fragility of chitosan, glycerol (Sigma Chemical Co., St. Louis, MO, USA) was added as 30% (w/w) of the total solid weight in solution [16]. Chitosan conduit was made according to the method described by others24 by gentle injection of the prepared solution into a home-made mold. The prepared conduit was 2 mm in external diameter, 1.8 mm in internal diameter and 10 mm in length. This internal diameter complies with optimal function in rat models [17].

2.3. Surgical procedure

Animals were anesthetized by intraperitoneal administration of ketamine–xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg). The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain [18]. The University Research Council approved all experiments.

Following surgical preparation in the normal control group, the left sciatic nerve was exposed through a gluteal muscle incision and after careful homeostasis the muscle was sutured with resorbable 4/0 sutures, and the skin with 3/0 nylon. In TC group, the left sciatic nerve was transected proximal to the tibio-peroneal bifurcation where a 7 mm segment was excised, leaving a 10 mm gap due to retraction of nerve ends. Proximal and distal stumps were fixed in the adjacent muscle with 10/0 nylon epineurial suture. No graft was interposed between the stumps. In the CHIT group, a 7-mm nerve segment was resected to produce a 10 mm nerve gap after retraction of the nerve transected ends. The gap was bridged using a chitosan conduit. Two 10/0 nylon sutures were used to anchor the graft to the epineurium at each end. The animals were anesthetized and euthanized with transcardiac perfusion of a fixative containing 2% paraformaldehyde and 1%glutaraldehyde buffer (pH 7.4) 4, 8 and 12 weeks after surgery.

2.4. Pulsed electromagnetic fields treatment

Following recovery from anesthesia, rats were randomly assigned to control or experimental groups. Pulsed electromagnetic fields treatment was performed based on a method described by others [10,11]. In brief, on days 1–5, each animal was placed in an all-plastic restrainer located between Helmholtz coils and treated for 4 h each day with the PEMF signal generator either activated (CHIT/PEMF group) or not activated (CHIT). PEMF was applied using paired Helmholtz coils (PHYWE, 06514, Germany) 30 cm in diameter, placed 15 cm apart. When connected to a signal generator (Funktiongenerator, PHYWE, Göttingen, Germany), these coils produced a magnetic field amplitude of 0.3 mT with a pulse duration of 20 msec, repeated at a pulse repetition rate of 2 Hz. The rise time was 0.85 msec, the fall time 0.68 msec.

2.5. Behavioral testing

Functional recovery of the nerve was assessed using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale for rat hind limb motor function [19]. Although BBB is widely used to assess functional recovery in spinal cord injured animals, however, it has been demonstrated that it could be most useful in assessment of never repair processes in peripheral nerve injuries [20]. Scores of 0 and 21 were given when there were no spontaneous movement and normal movement, respectively. A score of 14 shows full weight support and complete limbs coordination. BBB recordings were performed by a trained observer who was blinded to the