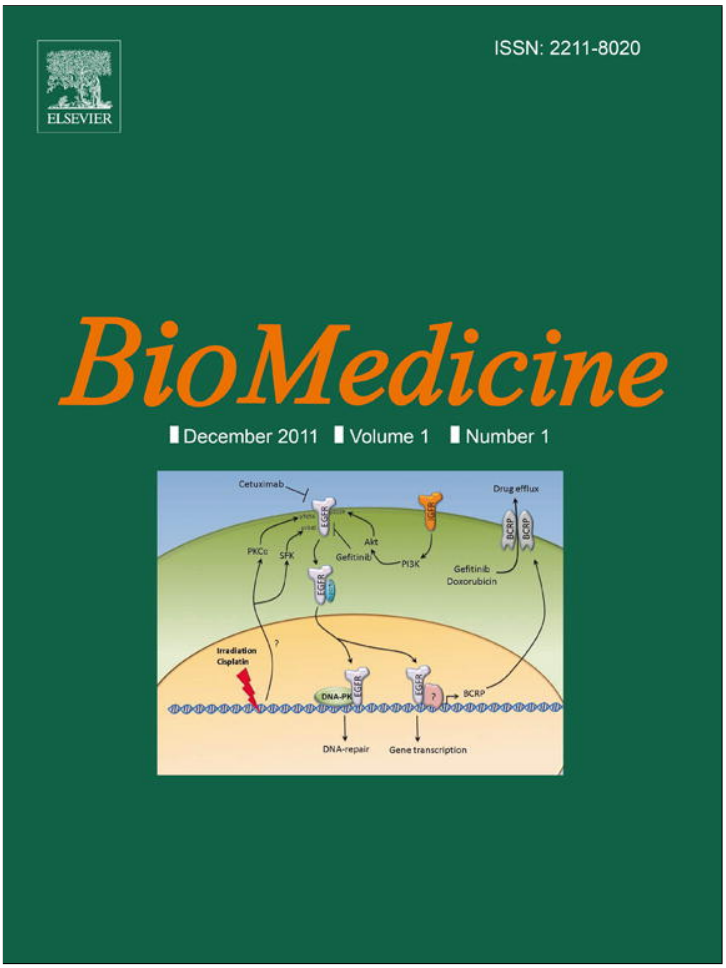

BioMedicine 1 (2011) 33–36

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Review article

Effects of electrical stimulation on peripheral nerve regeneration

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ARTICLE INFO

Article history:
Received 3 June 2011
Received in revised form
3 July 2011
Accepted 22 September 2011
Available online 13 November 2011

Keywords:
biomaterials
electrical stimulation
nerve repair

ABSTRACT

Over the past few years, my group has been investigating the effects of different parameters of electrical stimulation on nerve regeneration of a 10-mm gap of rat sciatic nerve created between the proximal and distal nerve stumps, which were sutured into silicone rubber chambers. In this review, I will introduce our work and share our experience with investigators who are interested in the fields of nerve regeneration and biomedical engineering.

1. Introduction

End-to-end and fascicular suture repair techniques are recommended for short nerve injury. However, in cases of extensive nerve injury, i.e., defects involving an irreducible gap between the injured proximal and distal stumps, a nerve graft or a nerve bridge is preferred. Donor nerves for grafting are often difficult to acquire; therefore, considerable research has been conducted on peripheral nerve repair using the nerve bridge technique [1–3]. A nerve bridge technique involves placing both ends of the injured nerve stumps into a tubular chamber, which helps guide growing nerve fibers along appropriate paths and enhances the precision of stump approximation. Regeneration of longer gaps can be achieved by prefilling the guidance chamber with chemical adjuncts such as neurite-promoting factors and neurotrophic factors, which can promote early peripheral nerve regeneration [4–6]. In addition to chemical adjuncts, physical adjuncts such as electrical stimulation (ES) have been used to recover lost function of injured nerve pathways in the peripheral nervous system.

Studies have demonstrated that a weak electric field can enhance neurite outgrowth in vitro [7,8] and in vivo [9,10]. Other studies, however, have reported that electric fields have no effect and in some cases a negative effect on nerve regeneration [11,12]. Similarly, discrepant findings have also been noted between studies that have adopted different stimulation frequencies and intensities. For example, Cheng et al found that pulse ES at 100 Hz could induce a relatively higher regenerated axonal density than electrical stimulation at 50 Hz [13]. However, Agnew et al found a positive correlation between the frequency of ES applied to a peripheral nerve and the severity of stimulation-induced neural damage [14]. In addition, the ideal duration that ES should be applied in patients with and in animal models of nerve injury has not been established. Furthermore, all of the aforementioned

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2211-8020/$ – see front matter
doi:10.1016/j.biomed.2011.10.006

studies that investigated the effect of ES on nerve regeneration in animal models focused on short nerve gaps. The inherent regenerative capacity of the nerve in animals could be so efficient over shorter gaps that the effects of ES may not be fully revealed. Therefore, animal models of nerve injury involving longer nerve gaps are needed to better understand the effects of ES on damaged nerves.

This review will introduce the effects of different frequencies, current intensities, and durations of ES on the regeneration of transected rat sciatic nerves that were reconnected using a silicone rubber nerve tube with a 10-mm gap.

2. Effects of ES frequency on sciatic nerve regeneration

It has been reported that ES can enhance peripheral nerve regeneration [15,16]. Based on the results of those studies, we are confident that ES produces bio-effects on nerve tissues. However, contradictory results have also been reported. Therefore, a dramatic and reproducible model with well-controlled experimental variations is necessary to clarify the role that electrical treatment plays on nerve regeneration.

The nature of the experiments, such as the type of ES used (DC or AC; constant or pulsed), the stimulation parameters, the sites for the placement of electrodes, and most importantly the length of the nerve gap, all affect the efficacy of ES on nerve regeneration. Considerable research has been conducted on nerve repair across a wide gap using entubulation techniques [17]; however, to the best of our knowledge, Cheng et al are the only researchers to have used conduit prostheses to investigate the influence of different ES frequencies on nerve regeneration [13]. In their study, histomorphometric evaluation revealed that ES frequency affected nerve fiber density. Unfortunately, the small nerve defect (7 mm in length) and the lack of electrophysiologic data hindered their ability to make a solid conclusion.

In a recent study, we used a silicone rubber conduit to repair a rat sciatic nerve defect measuring 10 mm in length and then stimulated the nerve with different electrical frequencies. Histological and electrophysiological techniques were used to determine whether ES could stimulate the regeneration of nerves. We found that ES significantly suppressed the formation of nerve cables across the nerve gap in the silicone rubber chamber in a dose-dependent manner. Our data showed that a frequency of 2 Hz resulted in generation of nerve cables across the gap in 86% of the subjects, that a frequency of 20 Hz stimulated nerve regeneration in 71% of the animals, and that 200 Hz resulted in regeneration of nerve cables across the gap in only 57% of the test subjects. In contrast, bridging cables were noted in all of the animals in the control group as well as in the ES group that received ES at a frequency of 1 Hz. These findings show that electrical treatment may interfere with the process of nerve regeneration. However, examination of muscle action potentials (MAPs) and morphology revealed that ES seems to exert a growth-promoting effect on regenerated nerves. Morphometric studies revealed that the regenerated nerves that received electrical treatment at a frequency of 2 Hz had a significantly shorter latency, a longer duration, a faster nerve conductive velocity (NCV), a smaller cross-sectional area, a larger axonal density, and a larger ratio of blood vessel area to total nerve than controls. Those findings indicate that electrical treatment can accelerate the maturation of regenerated nerves [18].

These results raise a number of questions. For example, how can the discrepant results be explained? In addition, how should “successful nerve regeneration” within a guidance tube be defined? We believe that both the percentage of regenerated nerves that successfully cross the gap as well as the maturity of nerve microstructure must be considered when assessing the recovery of regenerated nerves.

3. Effects of ES current intensity on sciatic nerve regeneration

Several investigators have tried to explain how application of cathode distal current enhances the regeneration of peripheral nerves. Sisken et al reported that direct current resulted in an increased number of neurotrophic factor receptors in chick embryos [19]. It has also been reported that proteoglycan-mediated adhesion of regenerating axons, which is necessary for neuronal cell growth, could be manipulated by direct current [20]. In addition, some studies showed that treatment with ES led to an increase in the expression of injury/regeneration-associated genes (growth-associated protein 43 and Tα1 tubulin) as well as neurotrophin brain-derived neurotrophic factor and its receptor trkB, factors that play important roles in the regeneration of nerve tissues [15,16].

We also found that animals exposed to ES had a larger mean number of axons, endoneurial area, total nerve area, blood vessel number, and blood vessel area than control animals, which indicates that ES accelerates the maturation of regenerated nerves [21]. In addition, regenerated nerves treated with ES, especially in the group that received 1 mA of direct current, had relatively shorter latency periods, larger amplitudes, larger MAP areas, faster NCVs, and more evidence of reinnervation of muscle fibers than controls. These results indicate that the transected nerves that received ES underwent adequate regeneration. We also found that the ability of ES to improve the function of regenerated nerves decreased as the current intensity increased. For example, animals that received ES at 4 mA had a significantly higher error rate than controls while crossing the grid runway in the kinematic gait analysis, a test that assesses individual limb motor functions [22]. Our results indicate, therefore, that excessive ES can hinder the functional recovery of regenerated nerves.

4. Effects of ES timing on sciatic nerve regeneration

It is generally assumed that ES should be applied shortly after nerve injury because ES-induced recovery can be facilitated by the body’s immune response to injury [23,24]. In addition, studies have shown that ES can also accelerate upregulation of brain-derived neurotrophic factor (BDNF) and trkB mRNA,

factors that support the development, maintenance, and plasticity of peripheral neurons [15,16]. Accordingly, delayed onset of ES should be less effective in promoting the recovery of regenerated nerves. However, those studies assessed ES only over a short gap. We wondered whether more severely injured animals require more time before ES can increase neuroplasticity. In one of our current studies [25], we found that the number of axons was significantly greater in rats that received ES at a frequency of 2 Hz and an intensity of 1 mA on Day 8 following nerve repair than in rats that did not receive ES. This result confirms that ES accelerates the maturation of regenerated nerves that successfully cross the gap and leads to improved sensorimotor function after peripheral nerve injury. In addition, we also found that application of a delayed ES could dramatically improve the recovery of regenerated nerve function. This beneficial effect was not seen when the same stimulation protocol was applied immediately after nerve repair. Specifically, we found that a delay of 7 days before the onset of ES significantly enhanced the formation of nerve cables across a wide nerve gap in the silicone rubber chamber. Seventy percent of the animals in that group had cables that grew across the gap whereas only 30% of the animals in the group that received ES on post-injury Day (PID) 1 exhibited such bridging cables. Nerve recovery after a delayed onset of ES was a surprising finding, as most studies have shown that early application of ES is effective at accelerating axonal regeneration, mainly by up-regulating the expression of growth-promoting factors such as BDNF [10,26]. However, in those studies the nerve gap was much shorter than that in our study. Therefore, we do not know whether more severely injured animals require more time after nerve repair before ES can increase neuroplasticity. In addition, when considering the onset of ES following nerve repair, it has to be kept in mind that early application of ES might result in side effects, such as exacerbation of the size of the lesion [27]. For example, Griesbach et al found that BDNF levels significantly increased in rats with a mild fluid-percussion injury (FPI) that were exercised from PID 14 to 20. In rats with moderate FPI, however, significant increases in BDNF were evident only in animals that were exercised from PID 30 to 36 [28]. Those results indicate that the time window for exercise-induced increases in BDNF is dependent on injury severity. Since both ES and exercise share some common mechanisms of action (i.e. increased expression of neurotrophin BDNF and its receptor trkB) [29], it is reasonable to assume that delayed ES should be more effective than immediate ES in promoting nerve regeneration across a large gap. Interestingly, we only found significant improvement in the rate of successful regeneration when ES was started after a delay of 7 days, but not when performed between Days 15 and 29 post-injury. This result again indicates the importance of the timing phenomenon for the effect of ES on growth-promoting factors [30].

It is also important to note that delayed ES not only increased the rate of successful regeneration, but also enhanced maturity of the neural components within the nerve cable [25]. Specifically, the number of myelinated axons that successfully grew across the 10-mm gap was twofold greater in the groups that received delayed ES than in the group that received immediate ES. In addition, the number of regenerated blood vessels was greater and the nerve areas were larger in the groups that received delayed ES than in the group that received immediate ES. Although we cannot explain the increased number of regenerated axons and blood vessels, our results indicate that delayed application of ES affects axonal and capillary growth in the regenerated nerves.

Our findings substantiate that the time course of ES is of importance for the final recovery after peripheral nerve injuries. A short delay in the onset of ES to injured nerves can significantly accelerate axonal regrowth and functional restoration, which are important factors for successful nerve regeneration.

5. Conclusion

This review demonstrates that ES has a dual effect: it can hinder the growth of regenerating nerves as well as promote their recovery. Safe stimulus protocols, therefore, are necessary. Otherwise, improper ES can irreversibly damage nerve tissue, retarding the process of nerve regeneration.

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