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Delayed repair of the peripheral nerve: A novel model in the rat sciatic nerve

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HIGHLIGHTS

- A novel, standardized delayed nerve repair model is established in rats.
- The longer the delay, the more challenging the model is for nerve regeneration. The choice of delay intervals can be tailored to meet specific requirements in future studies.
- Quantitative measurements and guidelines for the model development are described in details for reproducibility purposes.

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ABSTRACT

Peripheral nerve reconstruction is seldom done in the acute phase of nerve injury due to concomitant injuries and the uncertainty of the extent of nerve damage. A proper model that mimics true clinical scenarios is critical but lacking. The aim of this study is to develop a standardized, delayed sciatic nerve repair model in rats and validate the feasibility of direct secondary neurrorraphy after various delay intervals. Immediately or 1, 4, 6, 8 and 12 weeks after sciatic nerve transection, nerve repair was carried out. A successful tension-free direct neurorraphy (TFDN) was defined when the gap was shorter than 4.0 mm and the stumps could be reapproximated with 10-0 stitches without detachment. Compound muscle action potential (CMAP) was recorded postoperatively. Gaps between the two nerve stumps ranged from 0 to 9 mm, the average being 1.36, 2.85, 3.43, 3.83 and 6.4 mm in rats with 1, 4, 6, 8 and 12 week delay, respectively. The rate of successful TFDN was 78% overall. CMAP values of 1 and 4 week delay groups were not different from the immediate repair group, whereas CMAP amplitudes of 6, 8 and 12 week delay groups were significantly lower. A novel, standardized delayed nerve repair model is established. For this model to be sensitive, the interval between nerve injury and secondary repair should be at least over 4 weeks. Thereafter the longer the delay, the more challenging the model is for nerve regeneration. The choice of delay intervals can be tailored to meet specific requirements in future studies.

1. Introduction

In most clinical scenarios primary nerve reconstruction is not done in the acute phase of nerve injury due to concomitant injuries and the uncertainty of the extent of nerve damage. Secondary nerve repair takes place when the patient is stabilized from more vital injuries, when the local soft tissue conditions allow, and when the demarcation of damage along the nerve is clear. This poses a delay between the injury and nerve repair. The interval between nerve injury and surgery is one of the most crucial factors that affect the functional recovery, long-term delayed nerve repair always resulting in poor recovery (Sunderland, 1991; Samii et al., 1997, 2003; Aydin et al., 2004; Ma et al., 2007). The poor recovery is attributed to chronic denervation (prolonged denervation of distal nerve stumps and target muscles) and chronic axotomy (prolonged disconnection between neurons and target organs) (Fu and Gordon, 1995a,b; Sulaiman and Gordon, 2009; Xu et al., 2010; Gordon et al., 2011). The individual effect of chronic axotomy and chronic denervation on poor nerve function recovery is respectively studied by

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transferring a denervated proximal nerve stump to a freshly cut distal nerve stump (Fu and Gordon, 1995a) and by transferring a freshly cut proximal nerve stump to a denervated distal nerve stump (Fu and Gordon, 1995b). Chronic axotomy significantly reduces the ability of axons' regeneration and functional connection with target muscles (Fu and Gordon, 1995a), induces more neuronal death (Ma et al., 2001) and less motor axon regeneration (Xu et al., 2010). It was reported that less than 10% motoneurons regenerated into the nerve stump after one year denervation (Sulaiman and Gordon, 2000). Chronic denervation induces progressive deterioration of the intramuscular nerve sheaths resulting in blocking the pathways of new regenerating axons and forcing the regenerating axons grow outside the nerve sheaths (Fu and Gordon, 1995b). It was also deemed that the denervated target muscles lost the receptiveness of regenerating nerve axons (Aydin et al., 2004; Ma et al., 2007). Clinically, however, chronic axotomy is unavoidably accompanied with chronic denervation in the delayed nerve repair situation. So it is important to establish a proper delayed nerve repair model of experimental animal with both chronic axotomy and chronic denervation conditions to mimic the true clinical scenario for future scientific investigations.

The rat sciatic nerve injury and repair model is most commonly used due to the ease of surgery and the availability of many methods for assessing function (Wang et al., 2008a,b). Disproportionally there are only a few reports of using a delayed nerve repair model. Nearly all of the currently available sciatic nerve delayed reconstruction methods involve indirect methods such as autograft (Aydin et al., 2004), nerve transfer (Fu and Gordon, 1995a,b; Bain et al., 2001) or nerve conduit (Brown et al., 2002) to mitigate the gap resulting from nerve stump retraction that precludes direct secondary neurrorraphy.

These indirect repair models have some disadvantages: nerve transfer precludes the possibility of studying the combined effect of chronic axotomy and chronic denervation. Nerve conduits can be costly. Autograft is more time consuming and technically challenging, and inevitably sacrifices a sensory nerve resulting in additional pain, sensory loss and incision scars (Sunderland et al., 2004), which is not good for the animal welfare.

Saito and Dahlin (2008) and Saito et al. (2009) introduced a delayed sciatic nerve repair model in rats where direct secondary neurrorraphy was done. It seems hard to avoid nerve suture under tension after various delay intervals due to the obvious retraction of the distal nerve stump in this model. Suture under tension would prevent good nerve regeneration. Since the rats were sacrificed 10 days after nerve repair, studies of the functional effectiveness of nerve repair, such as electrophysiology, were not carried out in this report.

To the best of our knowledge, tension-free direct neurorraphy in the secondary nerve repair following various delay intervals has not been reported. We have successfully developed a novel delayed sciatic nerve repair model in rats by tagging the distal nerve stump to prevent retraction and validated the feasibility of tension-free direct secondary neurrorraphy after various delay intervals. The validity of the model was confirmed by signs of electrophysiological recovery 12 weeks after the secondary neurrorraphy. Furthermore, quantification of intraoperative characteristics was carried out to provide guidelines for the reproduction of this model and for the selection of a certain delay interval tailored to the need of a specific research study.

2. Materials and methods

2.1. Animals and group assignment

All procedures were approved by the Institutional of Animal Care and Use Committee. There were 58 female Lewis rats (Harlan Laboratories, Indianapolis, IN, USA) with body weight of 200 ± 5 g. All rats were housed in a temperature and humidity controlled room with 12 h light and 12 h dark cycles. The animals were offered standard chow and water ad libitum. All rats were housed at least two weeks before the surgery to adapt to the new environment. These rats were randomly divided into the following groups: group B0: sciatic nerve transection and immediate repair ($n = 8$); group B1: sciatic nerve transection and repair after 1 week delay ($n = 11$); group B2: sciatic nerve transection and repair after 4 week delay ($n = 10$); group B3: sciatic nerve transection and repair after 6 week delay ($n = 11$); group B4: sciatic nerve transection and repair after 8 week delay ($n = 11$); and group B5: sciatic nerve transection and repair after 12 week delay ($n = 7$). All rats from group B1 to group B5 underwent surgery A and surgery B as described below.

2.2. Surgery A (primary surgery)

All surgeries were done with the aid of an operating microscope under general anesthesia (80 mg/kg ketamine (Ketaset III; Fort Dodge Animal Health, Fort Dodge, IA, USA) and 5 mg/kg xylazine (AnaSed; Lloyd Laboratories, Shenandoah, IA, USA). The left sciatic nerve was exposed and isolated at the mid-thigh level using a dorsal-lateral approach with the left lower extremity at maximal extension position as shown in Fig. 1A and B. The left sciatic nerve was transected at 12 mm from the lower border of obturator tendon (Fig. 1C). The proximal nerve stump was turned around, embedded into the neighboring muscles and secured by one 9-0 monofilament nylon stitch (Ethilon, Ethicon Inc, New Brunswick, NJ, USA). The distal nerve stump was freed extensively so that it could be tagged at 10 mm from the lower edge of obturator tendon by two 9-0 monofilament nylon sutures to prevent retraction (Fig. 1D). The wound was closed in layers. Buprenorphine (Buprenorphine Hydrochloride Injection III, Hospira, Lake Forest, IL, USA), 0.05 mg/kg, was given subcutaneously once immediately after the surgery and daily in the first 3 postoperative days. Tylenol (Children's Mapap, Livonia, MI, USA), 30 ml per pint of drinking water, was given by oral administration 48 h before the surgery to acclimate rats with the flavor and was continued for the first 7 postoperative days.

2.3. Surgery B (secondary nerve repair surgery)

The sciatic nerve stumps were exposed again for the secondary nerve repair surgery after various delay intervals according to group assignment. The distal nerve stump was still well tagged by 9-0 stitches. The proximal nerve end was identified and freed from the neighboring muscle in which it was embedded (Fig. 1E). Intraoperative characteristics of the nerve were recorded as below. The proximal and distal nerve ends were approximated after freshening and directly sutured together with four to five 10-0 monofilament nylon stitches when possible. A successful direct nerve repair without tension was defined when the gap was no longer than 4.0 mm and the nerve repair with 10-0 stitches did not rip apart upon repeated maximal passive movements of the limb. The wound was closed in layers as described in surgery A. Tylenol and Buprenorphine were given as described in surgery A for postoperative pain control.

2.4. Intra-operative measurements during surgery B

The original transection position (Point A in Fig. 1C) was defined as 0 mm. The relative position of the distal nerve stump tip to the original transection position was measured and recorded. A position proximal to that level was denoted as positive, while a position distal to that level was denoted as negative (Point D in