Alpha-lipoic acid loaded in chitosan conduit enhances sciatic nerve regeneration in rat

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Abstract

Objective(s): To investigate the effect of topical administration of alpha-lipoic acid into chitosan conduit on peripheral nerve regeneration using a rat sciatic nerve transection model.

Materials and Methods: Forty five Wistar rats were divided into three experimental groups randomly. A 10-mm gap of sciatic nerve was bridged with a chitosan conduit following surgical preparation and anesthesia. In treatment group, the conduit was filled with 30 µl alpha-lipoic acid (10 mg/kg/bw). It was filled with 30 µl phosphate buffered saline solution in control group. In Sham group sciatic nerve was just exposed.

Results: The recovery of nerve function was faster in treatment group than in control, at 4 and 8 weeks after surgery (P-value<0.05). Conduction velocity was better in treatment group than in control group at 4 and 12 weeks (P-value<0.05). Recovery index was higher in treatment group than the control group, 8 weeks after surgery (P-value <0.05). Greater nerve fiber diameter, axon diameter, and myelin sheath thickness were observed in treatment group compared to control group at 8 and 12 weeks after surgery (P-value<0.05). The immunoreactivity of regenerated axons and myelin sheath in treatment group were far more similar to sham group.

Conclusion: Alpha-lipoic acid when loaded in a chitosan conduit could improve transected sciatic nerve regeneration in rat.

Keywords: Alpha-lipoic acid, Chitosan conduit, Rat, Sciatic nerve regeneration

Introduction

Return to normal function of peripheral nerve injuries is one of the most important aims in neurosurgery. Numerous surgical methods such as nerve graft and conduits filled with neurotrophic substances and cells have been successfully used for improvement of peripheral nerve regeneration (1). Trauma to peripheral nerve leads to acute myelinoaxonal degeneration in the distal area of the damaged nerve, macrophage infiltration, Schwann cell proliferation, and axonal regrowth (2).

It is known that nerve injury is associated with enhanced oxidative stress. Increase of the free oxygen radical levels and reduced activities of antioxidant enzymes are observed after nerve trauma (2-4). The positive effects of local administration of vitamin E and pyrroloquinoline quinone on peripheral nerve regeneration in rat sciatic nerve transaction model have been reported (5). Also, the neuroprotective and neurotrophic effects of intraperitoneal injection of ubiquinone (CoQ10) and Crocin on nerve regeneration in rat sciatic conduit model have been shown (6, 7).

Antioxidants such as acetyl-L-carnitine, vitamin E, and alpha-lipoic acid (LA) are used successfully in treatment of experimentally nerve crush injuries (4, 8, 9).

LA (1,2-dithiolane-3-pentanoic acid), a disulphide derivative of octanoic acid, is known to act as an efficient anti-oxidant (10). Several studies demonstrated that LA can decrease ischemia-reperfusion injuries in the cerebral cortex (11), heart (12), and peripheral nerve (13). Also, Senoglu et al showed the positive effects of intraperitoneal LA administration on sciatic nerve crush by measuring superoxide dismutase and catalase activities (4). However, the effect of LA on peripheral nerve regeneration in transaction model of injury has not been clarified.

The purpose of this study was to investigate the effect of topical administration of LA into chitosan conduit on peripheral nerve regeneration using a rat sciatic nerve transaction model. Assessment of nerve regeneration was based on functional (walking track analysis), electrophysiological measurement, muscle mass, histomorphometric, and immunohistochemistry.

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(Schwann-cell detection by S100 expression) criteria at 4, 8, and 12 weeks after surgery.

Materials and Methods

Animals
Forty five healthy adult male Wistar rats, weighing 220-250 g were randomized into three groups of 15 animals each. Each group was further subdivided into three subgroups of five animals each. Two weeks before and during the entire experiments, rats were maintained in groups of 5 per cage in a natural day/night cycle in a controlled ambient temperature (23±2°C) with ad libitum food and water. All procedures were carried out in accordance with the guidelines of the Ethics Committee (14) and were approved by the Urmia University Research Council, Urmia, Iran.

Grafting procedure and animal grouping
Under ketamine-xylazine (intra-peritoneal, ketamine hydrochloride 5%; 90 mg/kg and xylazine hydrochloride 2%; 5 mg/kg) anesthesia, surgical technique was done according to standard procedures (15). Briefly, in sham group, after exposing of the left sciatic nerve through a gluteal muscle incision, the muscle was sutured with absorbable 4-0 vicryl sutures, and the skin with 3-0 nylon. In control and treatment groups, following sciatic exposure the nerve was transected proximal to the tibio-peroneal bifurcation where an 8 mm segment was excised, leaving a gap about 10 mm due to retraction of the nerve ends.

The transected proximal and distal stumps were each inserted 2 mm into the 12 mm chitosan conduit and two 10-0 nylon sutures were placed at each end of the cuff to fix the tube in place. In treatment group the chitosan conduit was filled with LA solution (10 mg/kg/bw; prepared up to 30 µl with PBS solution, Sigma-Aldrich Chemie, Munich, Germany) and in control group the chitosan conduit was filled with 30 µl PBS solution (4). Sterile Vaseline was used to seal the ends of the tubes to avoid leakage. The surgical incision was closed as mentioned above.

The preparation and the efficacy of chitosan conduit on peripheral nerve regeneration in rat model have been described in our previous study (16). Briefly, chitosan solution was prepared by 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 hr. The resultant chitosan was filtered through a Whatman No. 3 filter paper. Again, to remove any undissolved particles the solution was filtrated through vacuum filtration. To overcome the undesired fragile character, glycerol (Sigma Chemical Co., St. Louis, MO, USA) was added as 30% (w/w) of the total solid weight in solution (17). Chitosan conduit was made by gentle injection of the prepared solution into a home-made mold. The conduit was 2 mm in internal diameter and 12 mm in length (16).

No drugs were administered during the postoperative period. The animals were anesthetized (described above) and euthanized by transcardial perfusion of a fixative containing 2% paraformaldehyde and 1% glutaraldehyde buffer (pH 7.4) at 4 (n= 5), 8 (n= 5), and 12 weeks (n= 5) after surgery.

Sciatic functional index (SFI)
Evaluation of SFI was done on one day before surgery and on 4, 8, and 12 weeks following surgery based on the work of Bain et al (18). After painting of hind paws with water soluble blue ink, rats immediately walked along an 8 × 80 cm corridor lined with white paper. The paw-prints were collected. Paw length and toe spread were measured. SFI was calculated for each animal by the following formula:

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\text{SFI} = \frac{-38.3\text{[(EPL-NPL)/NPL]} + 109.5\text{[(ETS-NTS)/NTS]} + 13.3\text{[(EIT-NIT)/NIT]} - 8.8}{38.3\text{[(EPL-NPL)/NPL]} + 109.5\text{[(ETS-NTS)/NTS]} + 13.3\text{[(EIT-NIT)/NIT]} - 8.8}
\]

PL is the distance from the third toe to its heel, TS is the distance from the first to the fifth toe, and IT is the distance from the second toe to the fourth toe on the experimental side (E) and the contralateral normal side (N) in each rat. SFI equal to -100 indicates significant impairment, whereas an SFI oscillating around 0 is considered to reflect normal function.

Electrophysiological measurements
At 4, 8, and 12 weeks after surgery, the electrophysiological studies were performed under general anesthesia (as described above) with Nacro bio system 320-3760 A trace 80 (Austin, Texas, USA). After exposing of sciatic nerve (both the operated side and non-operated side), single electrical pulses (at supramaximal intensity) were delivered via bipolar electrodes placed in turn at the proximal and distal trunk of the regenerated nerve cable and electromyography (EMG) was recorded by inserting an electrode into the belly of gastrocnemius muscle. After recording of EMG, differences in latency of EMG, the amplitude and the distance between the operated side and non operated side were measured. EMG, the amplitude and the distance between the proximal and distal sites of stimulation were measured to calculate the conduction velocity (19).

To remove variations between animals, the conduction velocity of the bridged nerve was expressed as a percentage of that on the intact side of each animal (% CVR) (20). The recovery index of EMG amplitude in all groups was calculated by the formula: recovery index = peak amplitude of the operated side/peak amplitude of the intact side (21).

Muscle weight measurement
Following electrophysiological assessments, the animals were euthanized and the gastrocnemius...