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Original research

Effect of in situ delivery of acetyl-L-carnitine on peripheral nerve regeneration and functional recovery in transected sciatic nerve in rat

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HIGHLIGHTS

- Long-term systemic administration of ALC increases the quantity and quality of nerve regeneration after primary nerve repair.
- A localized, effective and sustained delivery is crucial for a potentially broader use of ALC.
- ALC when loaded in a chitosan tube improved functional recovery of transected sciatic nerve in rat.
- This approach could deliver a superiour quality of reinnervation in a shorter period of time.

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ABSTRACT

The repair of peripheral nerve injuries is still one of the most challenging tasks and concerns in neurosurgery, plastic and orthopedic surgery. Effect of acetyl-L-carnitine (ALC) loaded chitosan conduit as an in situ delivery system of ALC in bridging the defects was studied using a rat sciatic nerve regeneration model. A 10-mm sciatic nerve defect was bridged using a chitosan conduit (CHIT/ALC) filled with 10 μL ALC (100 ng/mL). In control group (CHIT), the conduit was filled with the same volume of the phosphate buffered solution. The regenerated fibers were studied 4, 8, 12 and 16 weeks after surgery. The functional and electrophysiological studies confirmed faster recovery of the regenerated axons in ALC treated than control group ($P < 0.05$). The mean ratios of gastrocnemius muscles weight were measured. There was statistically significant difference between the muscle weight ratios of CHIT/ALC and CHIT groups ($P<0.05$). Morphometric indices of regenerated fibers showed number and diameter of the myelinated fibers in CHIT/ALC were significantly higher than in control group. In immuohistochemistry, the location of reactions to S-100 in CHIT/ALC was clearly more positive than CHIT group. ALC when loaded in a chitosan conduit resulted in improvement of functional recovery and quantitative morphometric indices of sciatic nerve.

1. Introduction

The repair of peripheral nerve injury is still one of the most challenging tasks and concerns in neurosurgery, plastic and orthopedic surgery. Nerve autograft remains the gold standard; however, there are several drawbacks such as sacrifice of functioning nerves, loss of sensation and mismatch between nerve and graft [1].

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 [2,3].

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 [4]. Nevertheless, the 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 [5,6].

Acetyl-L-carnitine (ALC) is a naturally occurring amino acid derivative that has both neuroprotective and antinociceptive effects. The mechanisms of action of ALC are not clear and are likely to be multifactorial, with effects on circulating neurotrophins and

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synaptic transmission influencing both nerve structure/function and patient perception of neuropathic symptoms [7]. It has also essential physiological roles in mitochondrial oxidative energy metabolism [8].It is a physiological peptide integral to mitochondrial aerobic glycolysis [9], and has inherent antioxidant properties [10], that increases neuronal binding of [11], and responsiveness to nerve growth factor (NGF) [12,13]. ALC shows a protective and regenerative action profile on the nervous tissue after traumatic injuries [14].

Long-term systemic administration of ALC increases the quantity and quality of nerve regeneration after primary nerve repair [15]. It has been demonstrated that neuroprotective effect of ALC is dose-dependent [16]. It is not known yet that this effect translates into increased target organ reinnervation. If so, ALC could offer great therapeutic potential in case of chronic neuropathies occurring in diseases such as diabetes [17] and HIV disease [18] where the problem is not merely sensory neuronal death [19,20], but also the inability of neurons to maintain long peripheral axons and to regenerate into the target tissues as a result of chronic metabolic insufficiency [18]. Although clinically safe, however, tolerance to ALC appears to be accompanied with mild, infrequent side effects, including insomnia, gastric irritation and vomiting, fishy smell of breath and body odor. A localized, effective and sustained delivery is crucial for a potentially broader use of ALC and to the best knowledge of the authors the literature lacks investigation on the topical effect of ALC on peripheral and it is remained to be studied.

The present study was designed to evaluate effects of ALC loaded chitosan conduit as an in situ delivery system of ALC in bridging the defects in rat sciatic nerve transection model.

2. Materials and methods

2.1. Experimental design

Eighty male White Wistar rats weighing approximately 290 g were divided into four experimental groups ($n = 20$), randomly: Transected control group (TC), sham-surgery group (Sham), chitosan control group (CHIT) and ALC group (CHIT/ALC). Each group was again subdivided into four subgroups of five animals each and surveyed in four time points of 4, 8, 12 and 16 weeks. Two weeks before and during the entire experiments, the animals were housed in individual plastic cages with an ambient temperature of $23 \pm 3\,^{\circ}C$, stable air humidity, and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water.

2.2. Preparation of chitosan conduit

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 h. The resultant chitosan solution was filtered through a Whatman No. 3 filter paper then vacuum filtration to remove any undissolved 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.23 Chitozan 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 [21].

2.3. Grafting procedure

Animals were anesthetized by intraperitoneal administration of ketamine-xylazine (ketamine hydrochloride 5%, 90 mg/kg and xylazine hydrochloride 2%, 5 mg/kg). The procedures were carried out based on the guidelines of the Ethics Committee of the International Association for the Study of pain [22]. The University Research Council approved all experiments.

Following surgical preparation in the sham-operation group (Sham) 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 the CHIT group the left sciatic nerve was exposed through a gluteal muscle incision and transected proximal to the tibio-peroneal bifurcation where a 7 mm segment was excised, leaving a gap about 10 mm due to retraction of nerve ends. Proximal and distal stumps were each inserted 2 mm into a chitosan tube and two 10/0 nylon sutures were placed at each end of the cuff to fix the tube in place and to leave a 10-mm gap between the stumps. The conduit was filled with 10 μL the phosphate buffered saline and sterile Vaseline was used to seal the ends of the tubes to avoid leakage. In the CHIT/ALC group the conduit was filled with 10 μL acetyl − L-carnitine (Sigma–Aldrich Chemie GmbH, Germany)solution (100 ng/mL) diluted with normal saline The animals were anesthetized (see above) and euthanized with transcardial perfusion of a fixative containing 2% paraformaldehyde and 1% glutaraldehyde buffer (pH 7.4) 4, 8, 12 and 16 weeks after surgery.

2.4. 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 [23]. 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 [24]. 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 experimental design. The testing was performed in a serene environment. The animals were observed and assessed within a course of a 4-min exposure to an open area of a mental circular enclosure. BBB scores were recorded once before surgery in order to establish a baseline control and again weekly thereafter to assess functional recovery during 16 weeks.

2.5. Functional assessment of reinnervation

2.5.1. Sciatic functional index (SFI)

Walking track analysis was performed 4, 8, 12 and 16 weeks after surgery based on Bain et al., 1989 [25]. The lengths of the third toe to its heel (PL), the first to the fifth toe (TS), and the second toe to the fourth toe (IT) were measured on the experimental side (E) and the contralateral normal side (N) in each rat. The Sciatic Function Index (SFI) in each animal was calculated by the following formula:

$$\mathrm{SFI} = -38.3 \times (\mathrm{EPL} - \mathrm{NPL})/\mathrm{NPL} + 109.5 \times (\mathrm{ETS} - \mathrm{NTS})/\mathrm{NTS} + 13.3 \times (\mathrm{EIT} - \mathrm{NIT})/\mathrm{NIT} - 8.8$$

In general, the SFI oscillates around 0 for normal nerve function, whereas around −100 SFI represents total dysfunction. The SFI was assessed based on the CHIT group and the normal level was

considered as 0. The SFI was a negative value and a higher SFI meant the better function of the sciatic nerve.

2.5.2. Static sciatic index (SSI)

SSI is a time-saving digitized static footprint analysis described by others [26]. A good correlation between the traditional SFI and the newly developed static sciatic index (SSI) and static toe spread factor (TSF), respectively, has been reported by others [26]. The SSI is a time-saving and easy technique for accurate functional assessment of peripheral nerve regeneration in rats and is calculated using the static factors, not considering the print length factor (PL), according to the equation:

$$SSI = [(108.44 \times TSF) + (31.85 \times ITSF)] - 5.49$$

Where:

$$TSF = (ETS - NTS)/NTS$$
$$ITSF = (EIT - NIT)/NIT$$

Like SFI, an index score of 0 was considered normal and an index of −100 indicated total impairment.

2.6. Electrophysiological measurement

After 16 weeks, following the track test, all animals were subjected to electrophysiological studies using Nacro bio system 320–3760 A trace 80 (USA). Under general anesthesia the left sciatic nerve was re-exposed by incision of the skin at the previous surgical site. Single electrical pulses (at supra maximal intensity) were delivered via bipolar electrodes placed in turn at the proximal and distal trunk of the regenerated nerve and electromyography (EMG) was recorded by inserting an electrode into the belly of gastrocnemius muscle. The latency and the amplitude of EMG were obtained. Also, the difference in latency of EMG was measured, and the distance between the proximal and distal sites of stimulation was measured to calculate the conduction velocity across the regenerated nerve. On the contralateral, right intact side of each animal, similar measurements were made for the determination of conduction velocity. The conduction velocity of the bridged nerve was expressed as a percentage of that on the intact side of each animal to cancel off variations between animals (% CVR).

The recovery index of EMG amplitude in all groups was calculated based on Suzuki et al. using the following formula:

Recovery index = Peak amplitude of the operated side/Peak amplitude of the intact side [27].

2.7. Muscle mass

Recovery assessment was also indexed using the weight ratio of the gastrocnemius muscles 16 weeks after surgery. Immediately after sacrificing of animals, gastrocnemius muscles were dissected and harvested carefully from intact and injured sides and weighed while still wet, using an electronic balance. All measurements were made by two independent observers unaware of the analyzed group.

2.8. Histological preparation and quantitative morphometric studies

Operated nerve was dissected from surrounding tissues and a segment including several millimeters proximal and distal to the graft was harvested. Graft middle cable of Sham, TC, CHIT and CHIT/ALC groups were fixed in 2.5 percent glutaraldehyde. The grafts were postfixed in OsO4 (2%, 2 h), dehydrated through an ethanol series and embedded in Epon. Samples were cut in 5 μm, stained with toluidine blue and examined under light microscopy. Morphometrical analysis was carried out using an image analyzing software (Image-Pro Express, version 6.0.0.319, Media Cybernetics, Silver Springs, MD, USA). Equal opportunity, systematic random sampling and two-dimensional dissector rules were followed in order to cope with sampling-related, fiber-location-related and fiber-size related biases [28].

2.9. Immunohistochemical analysis

In this study, anti-S-100 (1:200, DAKO, USA) was used as marker for myelin sheath. Specimens were postfixed with 4% paraformaldehyde for 2 h and embedded in paraffin. Prior to immunohistochemistry nerve sections were dewaxed and rehydrated in PBS (pH 7.4). Then the nerve sections were incubated with 0.6% hydrogen peroxide for 30 min. To block non-specific immunoreactions the sections were incubated with normal swine serum (1:50, DAKO, USA). Sections were then incubated in S-100 protein antibody solution for 1 h at room temperature. They were washed three times with PBS and incubated in biotynilated anti-mouse rabbit IgG solution for 1 h. Horseradish peroxidase-labelled secondary antibody was applied for 1 h. After that all sections were incubated with 3,3′- diaminobenzidine tetrahydrochloride chromogene substrate solution (DAB, DAKO, USA) for 10 min. The results of immunohistochemistry were examined under a light microscope.

2.10. Statistical analysis

Experimental results were expressed as means ± SD. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Model assumptions were evaluated by examining the residual plot. Results were analyzed using a factorial ANOVA with two between-subjects factors. Bonferroni test for pairwise comparisons was used to examine the effect of time and treatments. The differences were considered significant when $P < 0.05$.

3. Results

3.1. BBB recovery

In order to assess hind limb recovery the open field locomotor was used. Fig. 1 shows BBB scores compared to the baseline. All experimental groups, except for sham, showed the greatest degree of functional deficit one week after surgery. The ALC group showed

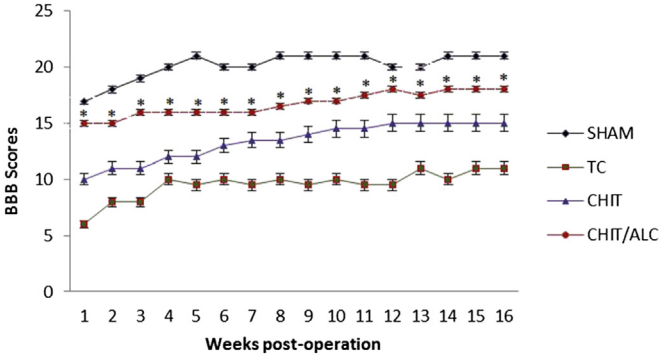

Fig. 1. BBB score for all experimental groups. Lopical administration of ALC with chitosan grafting gave better scores than in CHIT group. Standard error at each data point is shown with bars.

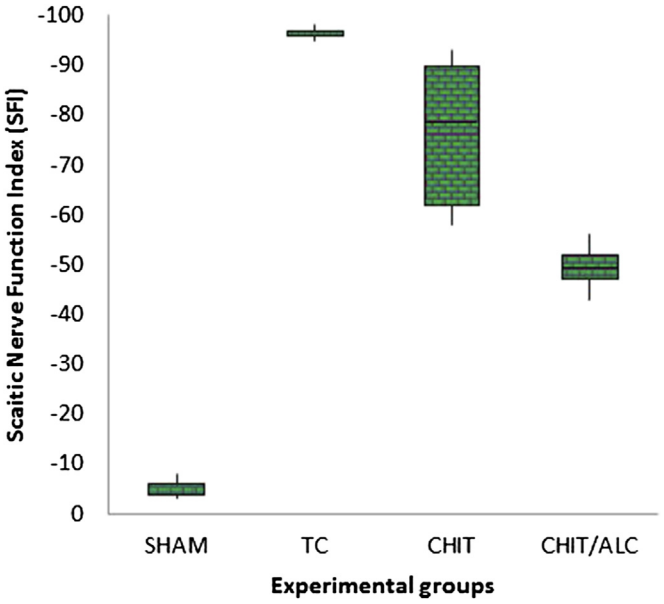

Fig. 2. Box-and-whisker plots of sciatic nerve function index values in each experimental group during the study period. Topical administration of ALC with chitosan tube grafting gave better results in functional recovery of the sciatic nerve than in CHIT group.

significant improvement in locomotion of the operated limb compared to the control group during the study period ($P < 0.05$).

3.2. Recovery of sciatic nerve function and reinnervation

3.2.1. SFI outcome

Fig. 2 shows sciatic function index (SFI) values in experimental groups. Prior to surgery, SFI values in all groups were near zero. After the nerve transection, the mean SFI decreased to −100 due to the complete loss of sciatic nerve function in all animals. Four weeks after surgery had performed mean SFI was −69.5 ± −2.39 in CHIT/ALC group, compared to −91.67 ± −3.22 in CHIT group. Eight weeks after surgery had carried out the improvement in SFI was observed in animals of CHIT/ALC group (−55.3 ± −2.25) that was significantly higher than CHIT (−75.2 ± −3.39) animals ($P < 0.05$).

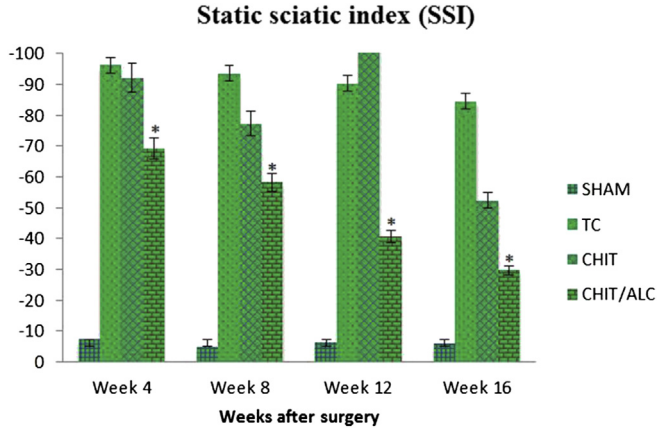

Fig. 3. Bar graph indicating static sciatic index (SSI) values in each experimental group during the study period. Local administration of ALC with chitosan grafting gave better results in functional recovery of the sciatic nerve than in CHIT group. Data are presented as mean ± SD. *$P < 0.05$ *vs* CHIT group.

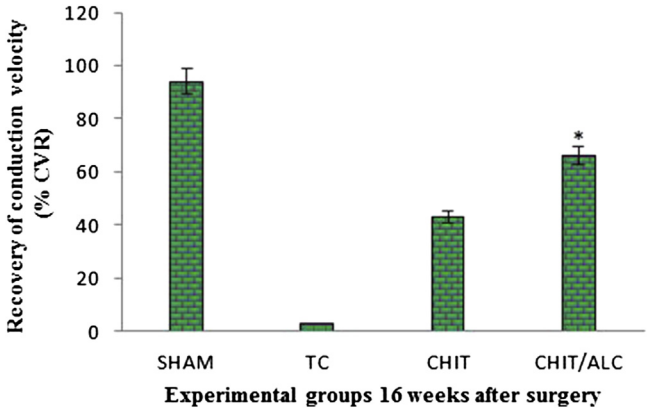

Fig. 4. Percentage recovery of conduction velocity in experimental groups. Data are presented as mean ± SD. *$P < 0.05$ *vs* CHIT group.

After 12 weeks, animals of group CHIT/ALC achieved a mean value for SFI of −38.4 ± −3.57, whereas in group CHIT, a mean value of −57.1 ± −4.10 was found. At the end of the study period, 16 weeks after surgery, animals of group CHIT/ALC achieved a mean value of −29.5 ± −3.75 for SFI of, whereas in group CHIT, a mean value of −46.3 ± −3.43 was found. The statistical analyses revealed that the recovery of nerve function was significantly faster in CHIT/ALC than CHIT group ($P < 0.05$) and, topically administered ALC promoted functional recovery.

3.2.2. SSI outcome

Changes in SSI were similar to those observed in SFI, indicating significant deficit following the sciatic nerve transection (Fig. 3). Changes in SSI were significant at weeks 4, 8, 12 and 16 weeks of recovery ($P < 0.05$). The contrasts indicated SSI values at week 16 to differ significantly from those obtained from control, a trend also noticed for SFI ($P < 0.05$).

3.3. Electrophysiology

Figs. 4 and 5 show nerve conduction velocity (NCV) along regenerated sciatic nerves in experimental groups. NCV in ALC treated animals was significantly higher than that in IOAG group ($P < 0.05$).

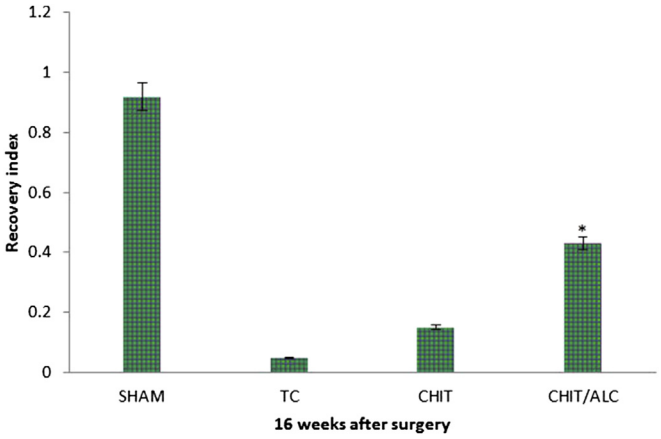

Fig. 5. Recovery index in experimental groups. Data are presented as mean ± SD. *$P < 0.05$ *vs* CHIT group.

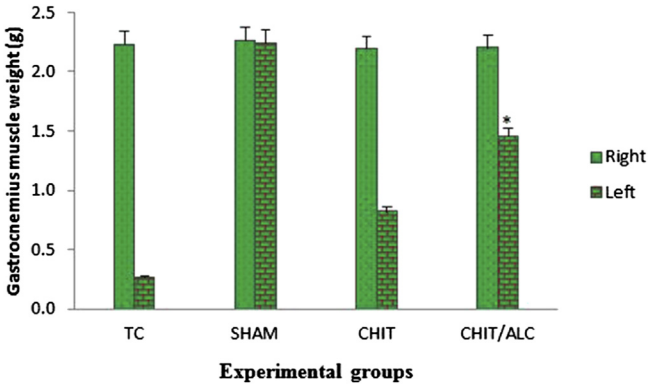

Fig. 6. Gastrocnemius muscle weight measurement. The gastrocnemius muscles of both sides (operated left and unoperated right) were excised and weighed in the experimental groups at 16 weeks after surgery. Data are presented as mean ± SD. *$P < 0.05$ vs CHIT group.

3.4. Muscle mass measurement

The mean ratios of gastrocnemius muscles weight were measured. There was statistically significant difference between the muscle weight ratios of CHIT/ALC and CHIT groups ($P < 0.05$). The results showed that in CHIT/ALC group muscle weight ratio was bigger than CHIT group and weight loss of the gastrocnemius muscle was ameliorated by local administration of ALC (Fig. 6).

3.5. Morphological findings

Table 1 shows quantitative morphometric analyses of regenerated nerves for each of the experimental groups. 4 weeks after surgery, CHIT/ALC group presented significantly greater nerve fiber, axon diameter and myelin sheath thickness compared to CHIT animals ($P < 0.05$). Although CHIT presented regeneration patterns, the morphometric indices in CHIT/ALC group both after 8, 12 and 16 weeks were better than CHIT.

Using Factorial ANOVA analysis with two between-subjects factors (Group × time); in the CHIT/ALC group number of nerve fibers and myelin thickness did not show significant difference between 8,12 and 16 weeks ($P > 0.05$). Increase in mean thickness of myelin sheath did not show statistical difference between 8, 12 and 16 weeks inside each group ($P > 0.05$). Mean thickness of myelin sheath from week 8 onward did not show significant difference between CHIT/ALC and Sham group ($P > 0.05$).

3.6. Immunohistochemistry

Immunoreactivity to S-100 protein was extensively observed in the cross sections of regenerated nerve segments. The expression of S-100 protein signal was located mainly in the myelin sheath. The axon also showed a weak expression indicating that Schwann cell-like phenotype existed around the myelinated axons (Fig. 7). In both groups, the expression of S-100 and the findings resembled those of the histological evaluations.

4. Discussion

Restoration of normal neurological function of transected peripheral nerve remains a great challenge in regenerative medicine and surgery. Entubulation neurorrhaphy is an excellent alternative to short interposition nerve grafts [29]. Selection of an appropriate method to evaluate functional recovery of nerve regeneration is extremely influential. Walking is a coordinated activity involving sensory input, motor response and cortical integration [30]. Therefore, walking track analysis (sciatic function index) is a comprehensive test. The results of the present study showed that ALC when loaded in a chitosan tube ended up a faster and significant improvement of functional recovery of the sciatic nerve throughout the study period.

Castaneda et al. [31], suggested that arrival of sprouts from the proximal stump at the distal nerve stump does not necessarily imply recovery of nerve function. Walking track analysis has frequently been used to reliably determine functional recovery following nerve repair in rat models [25,32]. Our results showed that moephometric indices were not significantly different between CHIT/ALC and CHIT groups after 8 weeks. In contrary, functional recovery occurred from week 8 to week 16 in CHIT/ALC supporting again this idea that selection of an appropriate method to evaluate functional recovery is crucial. This study also supports the idea that the walking track analysis (SFI) is more comprehensive and reliable than histomorphometric methods in peripheral nerve repair studies [31,33].

Recording wet muscle weight is a previously utilized alternative for motor target organ reinnervation [34–37]. In-vitro evidence suggests that ALC treatment improves the motor neuron activity, possibly acting as a neurotrophic factor [38], while evidence has been presented that suggests ALC may enhance functional muscle recovery in terms of attenuation of muscle atrophy, a reduction of foot drop, and increased toe spread [14].

Nerve conduction measurement is a direct evidence for the study of nerve transmission [39].The conduction velocity depends on the diameter of axons and the thickness of myelin sheath [40].

Table 1
Morphometric analyses of regenerated nerves for each of the experimental groups: values are given as mean ± SD.

Groups	Number of fibers				Diameters of fibers (μm)			
	4 Weeks	8 Weeks	12 Weeks	16 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks
Sham	8236 ± 371	8354 ± 430	8435 ± 382	8326 ± 340	12.05 ± 0.19	11.91 ± 0.14	12.03 ± 0.03	12.04 ± 0.05
TC	0	1155 ± 278	1276 ± 301	3812 ± 243	0	3.92 ± 0.51	4.15 ± 0.28	6.15 ± 0.25
CHIT	1661 ± 295	3256 ± 259	3788 ± 258	3876 ± 250	3.64 ± 0.83	8.06 ± 0.5	8.51 ± 0.70	8.95 ± 0.81
CHIT/ALC	3456 ± 224[a]	4467 ± 230[a]	6567 ± 271[a]	6678 ± 256[a]	8.46 ± 0.46[a]	9.78 ± 0.65[a]	10.78 ± 0.85[a]	10.80 ± 0.78[a]
Groups	Diameter of axon (μm)				Thickness of myelin sheath (μm)			
	4 Weeks	8 Weeks	12 Weeks	16 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks
Sham	7.04 ± 0.39	6.78 ± 0.35	7.06 ± 0.01	7.02 ± 0.19	2.57 ± 0.03	2.42 ± 0.01	2.50 ± 0.03	2.53 ± 0.04
TC	0	2.38 ± 0.36	2.44 ± 0.63	3.64 ± 0.47	0	0.81 ± 0.13	0.83 ± 0.02	1.25 ± 0.03
CHIT	2.65 ± 0.52	4.25 ± 0.44	4.85 ± 0.39	4.89 ± 0.44	0.48 ± 0.05	2.03 ± 0.38	2.19 ± 0.29	2.33 ± 0.27
CHIT/ALC	4.64 ± 0.47[a]	5.05 ± 0.58[a]	6.25 ± 0.41[a]	7.58 ± 0.39[a]	1.85 ± 0.06[a]	2.18 ± 0.27	2.33 ± 0.52	2.55 ± 0.50

N: number of fibers D: diameter of fibers (μm) d: diameter of axon (μm) T: thickness of myelin sheath (μm).
[a] Results were significantly different from CHIT group ($P < 0.05$).

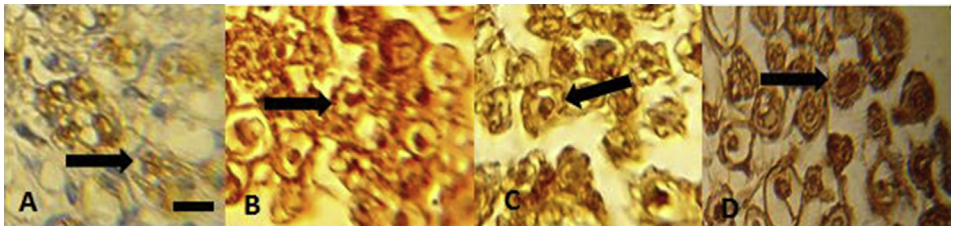

Fig. 7. Immunohistochemical analysis of the regenerated nerves 16 weeks after surgery from (A) middle cable TC, (B) CHIT, (C) CHIT/ALC and (D) SHAM. There is clearly more positive staining of the myelin sheath-associated protein S-100 (arrows) within the periphery of nerve, indicating well organized structural nerve reconstruction in ALC treated nerve compared to that of the CHIT. Scale bar: 10 μm.

The results of the present study showed significantly different conduction velocity between the ALC treated animals and CHIT bridged regenerated sciatic nerves, therefore, the CHIT conduit in combination with ALC could be assumed as a safe nerve guide with no nerve conduction interference.

As the posterior tibial branch of the sciatic nerve regenerates into the gastrocnemius muscle, it will regain its mass proportional to the amount of axonal reinnervation [41,42]. In the present study 16 weeks after surgery the muscle mass was found in both experimental groups. However, CHIT/ALC group showed significantly greater ratios of the mean gastrocnemius muscle weight than CHIT group indicating indirect evidence of successful end organ reinnervation.

In the histological studies, quantitative morphometrical indices of regenerated nerve fibers showed significant difference between CHIT and CHIT/ALC groups indicating beneficial effect of topical ALC on the nerve regeneration.

In immunohistochemistry the expression of axon and myelin sheath special proteins was evident in both groups which indicate the normal histological structure. The location of reactions to S-100 in CHIT/ALC group was clearly more positive than CHIT group further implying that both regenerated axon and Schwann cell-like cells existed and were accompanied by the process of myelination and the structural recovery of regenerated nerve fibers.

Beneficial effects of long-term systemic administration of ALC on the quantity and quality of nerve regeneration have been documented [15]. It has been suggested that long term use of ALC is not always well tolerated and infrequently has ended up side effects, including insomnia, gastric irritation and vomiting and odorous body [7]. Seeking novel techniques of ALC administration and to avoid drawback of long-term administration of ALC, we have shown in this proof of study that topical application of ALC at the site of transected sciatic nerve could improve functional recovery and target organ reinnervation in rat.

Neuroprotective properties of systemic ALC have been shown to persist even when ALC treatment is commenced up to 24 h post injury [16]. ALC increases the expression and affinity of neurotrophin receptors for nerve growth factor (NGF) and it has been reported that NGF has greatly improved muscle contractility, muscle weight and histological morphometric tests in neurotised soleus muscle [34] regarding neuro-trophic role of NGF on sensory neurons [43]. NGF has also been shown to enhance motor nerve conduction velocity after sciatic nerve division and repair via a conduit [44]. While repairing an injured nerve the results of this study suggests that topical ALC treatment would be of clinical benefit. It has recently been demonstrated that ALC treatment virtually eliminates sensory neuronal death after peripheral axotomy in vivo [45]. ALC significantly enhances the regenerative capacity of neurons that survive peripheral nerve trauma in addition to its known neuroprotective effects [46].

However, a topical formulation that provides the same neurotrophic stimulus would be ideal because of no long term systemic complications and time consuming limitations. Entubulation neurorrhaphy using ALC loaded chitosan tube as an in situ delivery system of ALC in bridging the defects could be considered as an excellent alternative to short interposition nerve grafts. The use of a chitosan tube seems to have several distinct advantages for the treatment of transected peripheral nerves because it is inert and does not induce extensive scarring or degeneration after implantation, available and easily performed.

5. Conclusion

In conclusion, this study demonstrated that ALC when loaded in a chitosan tube improved functional recovery of transected sciatic nerve in rat. Supported by previous findings, the results from our present study would imply that the final outcome of both motor and sensory regeneration and reinnervation following repair of a peripheral nerve and immediate topical application of ALC may be of clinical benefit. There are reasonable grounds to believe that this approach could deliver a superior quality of reinnervation in a shorter period of time, compared to repair without immediate topical adjuvant treatment.

Ethical approval

The study was approved by the ethics committee for animal experimentation by the Faculty of Veterinary Medicine, Islamic Azad University-Urmia Branch.

Sources of funding

None.

Author contribution

Mohammad Reza Farahpour: Study design, surgical procedures and writing.

Sina Jangkhah: Data collection and data analysis.

Conflicts of interest

None.

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