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Neuroprotective Effects of Acetyl-L-Carnithine in Experimental Chronic Compression Neuropathy. A Prospective, Randomized and Plasebo-Control Trials

Kadir KOTİL[1]
Mehmet KIRALİ[2]
Mustafa ERAS[3]
Turgay BİLGE[4]
Hafize UZUN[5]

[1,3,4,5] Haseki Educational and Research Hospital, Neurosurgery Clinic, İstanbul, Turkey
[2] University of Istanbul, Department of Biochemistry, Istanbul, Turkey

ABSTRACT

OBJECTIVE: We designed this experimental study to examine the potential positive influences of the acetylated derivative of acetyl-L-carnithine, an endogenous substance present in the nervous system, on chronic compression neuropathy. This is the first study ever published on the medical treatment of experimental chronic compression neuropathy.

MATERIALS AND METHODS: Five groups composed of 5 rats each were used in the study.

Group 1: The control group, in which a 1 cm-long segment proximally from the bifurcation point of the right sciatic nerve of each rat was excised, accompanied by removal of the right soleus muscle. Group 2: The compression neuropathy model group, in which the right sciatic nerve of each rat was compressed for 30 days. Group 3: The right sciatic nerves were compressed for 30 days, followed by decompression and assessment on the 60th day. Group 4: The right sciatic nerves were compressed for 30 days, followed by decompression and acetyl-L-carnithine administration between days 30 and 60. Group 5: The right sciatic nerves were compressed for 30 days, followed by acetyl-L-carnithine administration from day 30 to 60 without decompression. The study continued with the rats in the other 3 groups. Rats in the 3rd group were treated with decompression only and kept for another 1 month. Rats in the 4th group received acetyl-L-carnithine at a dose of 20 mg/kg/day intraperitoneally for 1 month after decompression, whereas rats in the 5th group received only intraperitoneal acetyl-L-carnithine at a dose of 20 mg/kg/day without decompression. Like the rats in groups 1 and 2, these rats were also sacrificed with ether overdose, with their right sciatic nerves and soleus muscles being excised for histopathological examination and weighing, respectively.

CONCLUSION: In our study, it was found that decompression significantly improves the recovery rate of peripheral nerve as compared with that without decompression, and that acetyl-L-carnithine coadministered with decompression enhances clinical and histopathological recovery. In addition, the use of silicon tubes in such experiments was found to be likely to have prominent advantages.

KEY WORDS: Acetyl-L-carnithine, Chronic nerve compression, Neuroprotective effects

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Correspondence address:
Kadir KOTİL
E-mail: kadirkotil@superonline.com

INTRODUCTION

Whether ischemic or mechanic factors are more to blame in the generation of compression neuropathy is still a matter of debate in the experimental literature. It is widely accepted that both are important in the pathophysiology of compression neuropathy, and moreover, they act additively. The pathophysiology of compression neuropathy is yet incompletely understood. In the model of Mackinnon, the earliest abnormality observed is the disruption of blood-brain barrier. This disruption in the blood-brain barrier precedes any electrophysiological or light microscopic alteration. The disruption of the blood-brain barrier leads to an increase in the endoneural fluid pressure and edema within the nerve (1-3). As a result of the edema, fibrous tissue replaces the neural tissue. Gelberman and Lundborg (21) proposed a similar pathophysiologic change in acute nerve compression.

ALC (γ-amino-β-hydroxy-butyrobetaine) is an acetylated derivative of carnithine. It is an endogenous substance present in the nervous tissue (7). However, its precise role in the central nervous system (CNS) functions is yet unclear. ALC is also found in the main biochemical pathways, such as the acetyl-CoA synthesis, where it stimulates the energy metabolism in mitochondria, and the modification of neural membrane phospholipid dynamics (7, 18, 33). ALC can also be utilized in the synthesis of acetyl-L-choline, which has a similar chemical structure to that of acetylcholine. The biochemical influences and electrophysiological findings of ALC suggest that it can play role in the cholinergic system. Recent studies have suggested that ALC also increases the number of nerve growth factor (NGF) receptors in the plasma membranes of targeted cells under the influence of NGF itself. Additional behavioral and clinical studies have demonstrated that ALC treatment improves the performances of old rats and humans in psychological tests (10, 36). The purpose of our study was to probe in 5 different rat groups whether ALC contributes to the peripheral nerve regeneration, and to examine the effectiveness of a silicon tube model, which is a novel model in nerve compression studies.

MATERIALS AND METHODS

A total of 25 male adult rats weighing 180-200 grams were used. The subjects were obtained from the Basic Sciences Animal Laboratory Facility in Cerrahpaşa Faculty of Medicine in Istanbul University following the approval of the Ethics Committee. The rats were kept in cages and fed with standard laboratory chow and tap water ad libitum. All rats had free access to tap water. The procedures involving animals were in conformity with national laws for the Care and Use of Laboratory Animals (1985).

The rats had been subjected to sleep-wake cycles for 7 days prior to surgery. Forty rats were assigned to five groups. The rats were treated with great care not to harm any physiological and biological structures during the experiments.

The animals were randomly distributed to experimental and control groups using simple randomization by computer generated sequences. They were then coded and assigned to the groups before the experiment.

Biochemical features were studied by the researchers in a double blind pattern.

Group 1: The control group, in which a 1 cm-long segment proximally from the bifurcation point of the right sciatic nerve of each rat was excised, accompanied by removal of the right soleus muscle.

Group 2: The compression neuropathy model group, in which the right sciatic nerve of each rat was compressed for 30 days.

Group 3: The right sciatic nerves were compressed for 30 days, followed by decompression and assessment on the 60th day (Table I)

Group 4: The right sciatic nerves were compressed for 30 days, followed by decompression and ALC administration between days 30 and 60 (Table II)

Group 5: The right sciatic nerves were compressed for 30 days, followed by ALC administration from day 30 to 60 without decompression (Table III).

Anaesthesia: Ketamine hydrochloride was administered intraperitoneally at a dose of 100 mg/kg to each rat for general anesthesia. All of the rats were used in this study and there were no exclusion criteria.

Surgery: Following the induction of anesthesia, a 3 cm-long transverse incision was made in each rat over the right gluteal muscle in prone position. The gluteal muscle was shifted laterally and a 2 cm-long segment of on the proximal side of the bifurcation of right sciatic nerve was dissected circumstantially (29). A silicon tube with an inner diameter of 1.3 mm

Table I: A statistically significant difference was found between the right soleus muscle weights of rats in the groups I and IV (p<0.0016).

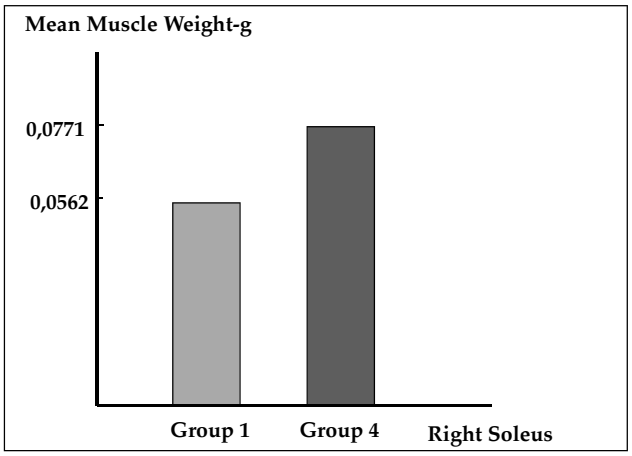


Table II: A statistically significant difference was found between groups II and IV (p<0.008).

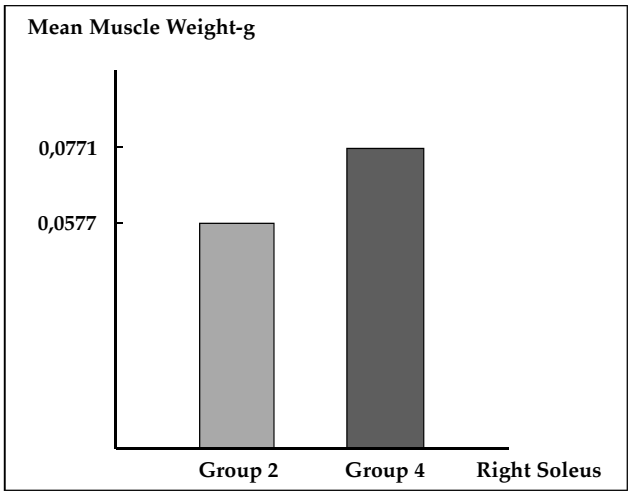
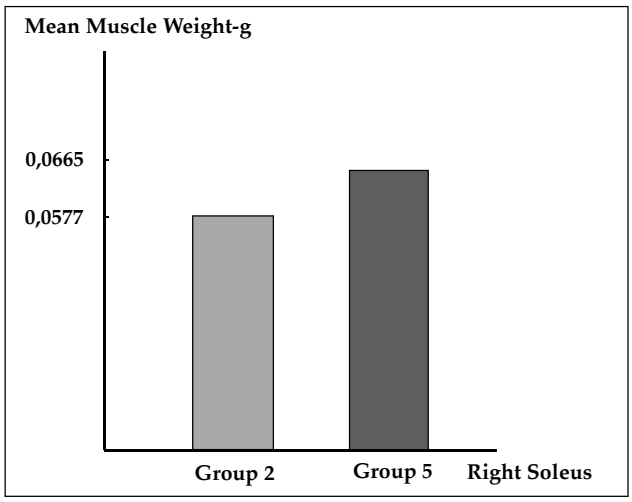


Table III: A statistically significant difference was found between groups II and V (p<0.008).



was incised longitudinally on one side and cautiously placed around the nerve. To warrant that the tube would not be dislocated spontaneously, it was sutured on both ends with a couple of 6/0 prolene stitches (39, 44). Following hemostasis, the muscle and skin layers were sutured accordingly. Upon awakening, each rat was observed to verify that it could move both legs.

Following the initiation of compression, the rats were kept in individual cages for 1 month for the development of chronic compression neuropathy. At the end of 1 month, rats in the groups with and without compression were sacrificed with ether anesthesia, and their right sciatic nerve samples were taken via excision for pathologic examination. Both soleus muscles in each rat were excised and weighed with sensitive scales. The purpose of weighing both muscles in each rat was to measure the difference between the right and left soleus muscles and to provide more objective comparisons between groups.

The study continued with the rats in the other 3 groups. Rats in the 3rd group were treated with decompression only and kept for another month. Rats in the 4th group received ALC at a dose of 20 mg/kg/day intraperitoneally for 1 month after decompression, whereas the rats in the 5th group received only intraperitoneal ALC at a dose of 20 mg/kg/day without decompression. Like the rats in groups 1 and 2, these rats were also sacrificed with ether overdose, with their right sciatic nerves and soleus muscles being excised for histopathological examination and weighing, respectively.

RESULTS

In light of these results, we can conclude that nerve decompression alone does not provide sufficient nerve regeneration in the early phase, and that the ALC administration as adjuvant treatment augments the regenerative process. On the other hand, statistical comparison of the 3rd (decompression) and the 4th (decompression + ALC) groups revealed no significant difference.

The absence of a statistically significant difference between the 3rd (decompression) and the 5th (compression+ALC) groups indicates that decompression alone is not more valuable than the drug administration without decompression.

One reason for the lack of a statistically significant difference between the 2nd (compression) and the 3rd (decompression) groups may be the

insufficiency of nerve regeneration within 30 days to increase the muscle weight. There was a statistically significant difference between the 2nd (compression) group and the group with compression for 60 days and ALC administration beginning on the 30th day ($p<0.008$). This indicates that positive results may be obtained with ALC administration alone, even in absence of nerve decompression.

The Pathological Results

After obtaining an 1 cm-long nerve specimen proximally from the bifurcation point of right sciatic nerve in each rat, every specimen was fixed with parafin, and stained with both Hematoxylen-Eosin (HE) and Toluidine Blue (TB). The pathological results are discussed under the figure (Figure 1-8)

Statistical Analyses: The 5 groups were analyzed using the Kruskal-Wallis test. Comparisons of the left soleus muscles innervated by the left sciatic

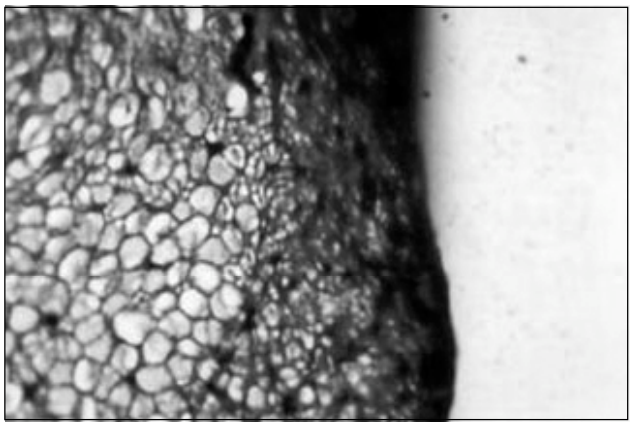


Figure 3: After compression for a month, tightening and irregularity of the axons underneath the perinerium and nerve diameter reduction are noted. TB x 10.

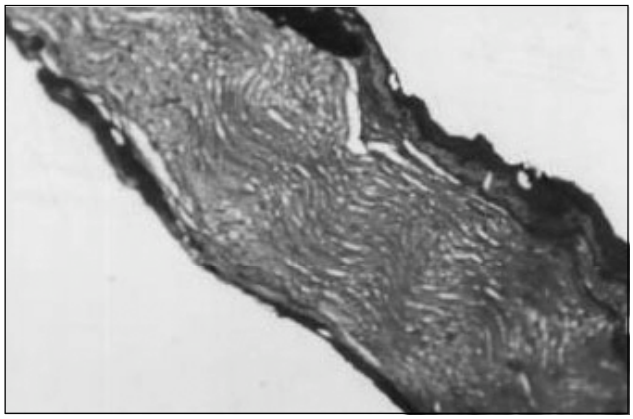


Figure 1: Longitudinal section of the rat sciatic nerve at the end of the compression for one month reveals axonal tightening under the perineurium. The nerve diameter is narrowed. Toluidine blue x 7.

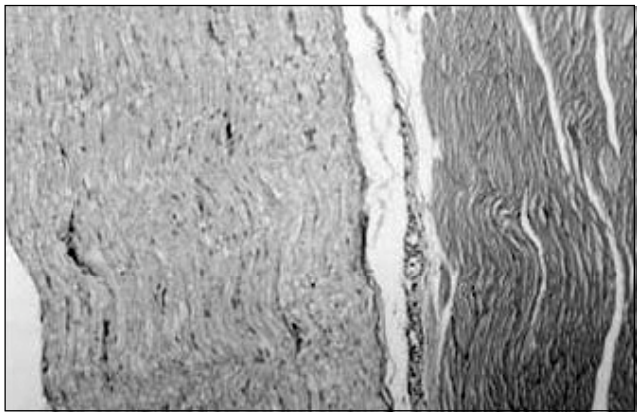


Figure 4: Sciatic nerve after decompression. The tightening under the perinerium is recovered. HE x 10.

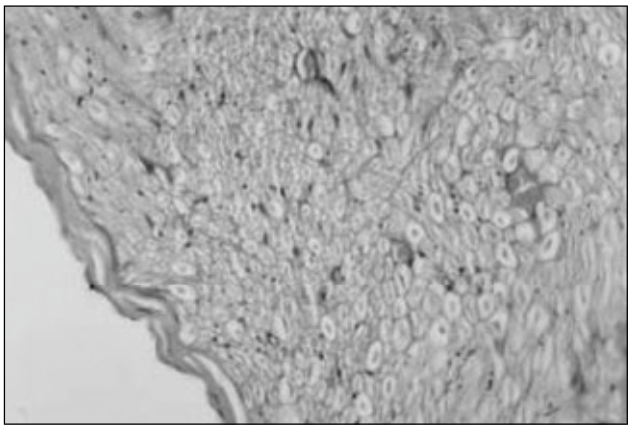


Figure 2: Compressed rat sciatic nerve. It is of note that the nerve fibers underneath the perinerium in this figure are tightened. HE x 10.

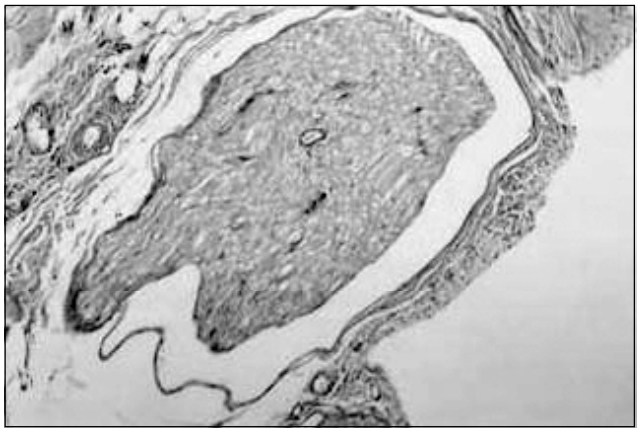


Figure 5: Rat sciatic nerve following decompression. No histological abnormality is found. HE x 7.

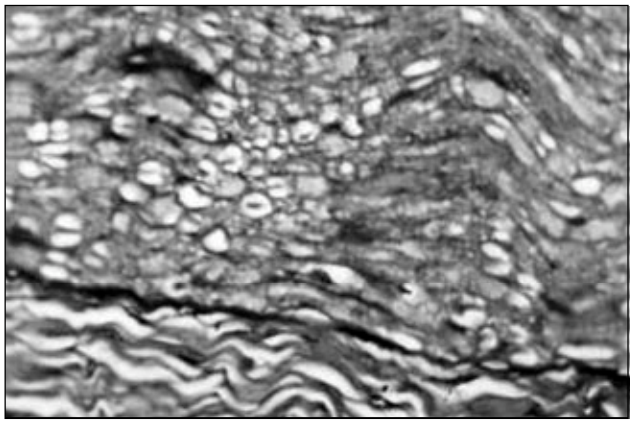


Figure 6: Sciatic nerve after both decompression and drug administration. Longitudinal section reveals regular axons with normal diameter underneath the perinerium.

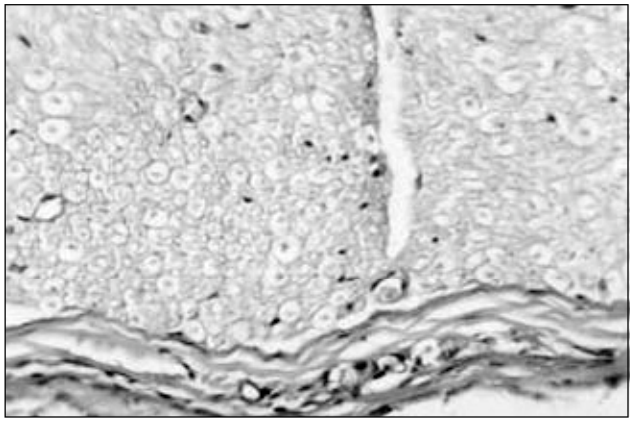


Figure 7: Rat sciatic nerve with both decompression and ALC administration. No tightening of the nerve fibers underneath the perinerium is present. Histologically, the nerve has a normal appearance in transverse section. HE x 10.

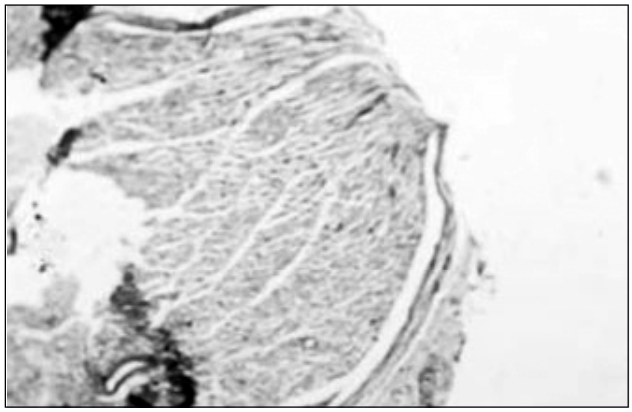


Figure 8: Sciatic nerve after drug administration without decompression. Transverse section displays alleviation of the nerve fiber tightening underneath the perineural connective tissue, with occasional restoration of the fibers. Toluidine blue x 7

nerves that were not manipulated (i.e., compressed, or decompressed) revealed no statistically significant difference. On the other hand, the right soleus muscles that were surgically manipulated differed significantly depending on the nature of the manipulation.

In the next step, the Mann-Whitney test was employed to probe the pairs that were significantly different. While neither the 2nd (compression group) nor the 3rd (decompression group) group was found to have a statistically significant difference when compared with the 1st (sham) group, the group with decompression and ALC administration (group 4) was significantly different than the 1st ($p<0.016$).

No statistically significant difference was presented between the 2nd (compression) and the 3rd (decompression) groups, whereas a significant difference was found between the 2nd and the 4th (decompression + ALC) groups ($p<0.008$).

DISCUSSION and CONCLUSION

The pathophysiology of compression neuropathy is incompletely understood, but has been suggested to be both vascular and mechanical (3, 6, 9, 37). A number of animal models have been developed in order to examine the pathophysiological and morphological alterations taking place during nerve compression. The segmental changes in myelin and the blood-brain barrier alterations have been described in detail. The chronic compression models have been less defined. Foreman et al (16) have studied chronic progressive nerve compression in 3-5 week-old rabbits. Such a developmental model is irrelevant, as adaptive changes may happen in young animals. Winding strings around nerves, and tying the nerve with arterial layers are indeed nerve degeneration and regeneration models.

Weisl and Osborne (35) have followed rats for 5 weeks after compressing their sciatic nerves with double-sylastic tubes (the compression diameter being 50% of the sciatic nerve in each rat). This study demonstrated changes same as those observed in human studies after nerve compression, but is not a chronic compression study as the compression diameter is small. In their study on Sprague-Dawley rats weighing 250-300 g, Mackinnon et al (32) have demonstrated histologically severe damage in groups with 0.6 and 0.9 mm compression diameters (50% and 75% of nerve diameter, respectively). The groups with 1.1 and 1.5 mm compression diameters have been found to be ideal for chronic compression

ABSTRACT OF THE CASE ANALYSES

	CASES					
	INCLUDED		EXCLUDED		TOTAL	
	N	percent	N	percent	N	percent
L.soleus * group	25	100.0%	0	.0%	25	100.0%
R.soleus * group	25	100.0%	0	.0%	25	100.0%

Report

	Group	Left soleus	Right soleus
Sham group	average	.056440	.056200
	N	5	5
	Std. Deviation	.0038753	.0039166
Compression- control	average	.061040	.053740
	N	5	5
	Std. Deviation	.0094124	.0089497
Decompression	average	.071600	.074220
	N	5	5
	Std. Deviation	.0135654	.0138366
Decompression + ALC	average	.075000	.077140
	N	5	5
	Std. Deviation	.0175153	.0148927
Compression + ALC	average	.073720	.066540
	N	5	5
	Std. Deviation	.0029978	.0072241
	average	.067560	.065568
	Total	25	25
	Std. Deviation	.0125707	.0135867

Kruskal-Wallis Test

Index

	Group	N	Average
Left soleus	sham group	5	6.40
	compression control	5	9.60
	decompression	5	14.80
	decompression + ALC	5	16.20
	compression + ALC	5	18.00
	Total	25	
Right soleus	sham group	5	6.40
	compression control	5	6.70
	decompression	5	17.50
	decompression + ALC	5	19.10
	compression + ALC	5	15.30
	Total	25	

Statistic tests (a,b)

	Left soleus	Right soleus
Chi-Square	8.640	13.492
Df	4	4
Asymp. Sig.	.071	.009

a Kruskal Wallis Test

b Chanceable Group: group

Mann-Whitney Test

Index

	Group	N	Average	Total
Left soleus	**Sham group**	5	3.60	18.00
	Decompression + ALC	5	7.40	37.00
	Total	10		
Right soleus	Sham group	5	3.20	16.00
	Decompression + ALC	5	7.80	39.00
	Total	10		

Statistic tests (b)

	Left soleus	Right soleus
Mann-Whitney U	3.000	1.000
Wilcoxon W	18.000	16.000
Z	-1.984	-2.402
Asymp. Sig. (2-tailed)	.047	.016
Exact Sig. [2*(1-tailed Sig.)]	.056(a)	.016(a)

Mann-Whitney Test

Index

	Group	N	Average	Total
Left soleus	Compression- control	5	4.20	21.00
	Decompression + ALC	5	6.80	34.00
	Total	10		
Right soleus	Compression- control	5	3.00	15.00
	Decompression + ALC	5	8.00	40.00
	Total	10		

Statistical Tests (b)

	Left soleus	Right soleus
Mann-Whitney U	6.000	.000
Wilcoxon W	21.000	15.000
Z	-1.358	-2.611
Asymp. Sig. (2-tailed)	.175	.009
Exact Sig. [2*(1-tailed Sig.)]	.222(a)	.008(a)

Mann-Whitney Test

Index

	Group	N	Average	Total
Left soleus	Compression – control	5	3.80	19.00
	Compression + ALC	5	7.20	36.00
	Total	10		
Right soleus	Compression – control	5	3.00	15.00
	Compression + ALC	5	8.00	40.00
	Total	10		

Statistical Test (b)

	Left soleus	Right soleus
Mann-Whitney U	4.000	.000
Wilcoxon W	19.000	15.000
Z	-1.776	-2.611
Asymp. Sig. (2-tailed)	.076	.009
Exact Sig. [2*(1-tailed Sig.)]	.095(a)	.008(a)

models. The silicon tubes with 1.5 mm inner diameter have been shown to be the most appropriate compressors (32, 38). In our study, we have found that the silicon tube with 1.3 mm inner diameter is the most appropriate one regarding avoidance of nerve trauma or damage, and can be used for the true compression model. We suppose that this study may be a pioneer for studies of this kind. For rats, the ideal duration of this compression model can be 1 month. With the model developed by Mackinnon et al (32), which has been accepted as the most appropriate compression neuropathy model (compression of the rat sciatic nerve with a sylastic tube that is 10 mm in length and 1.3 mm in inner diameter), chronic compression neuropathy that can be pathologically diagnosed 30 days later had been induced. The earliest pathologic change observed in Mackinnon's model is disruption of the blood-nerve barrier. Disruption of the blood-nerve barrier precedes any other pathology observed with the electron or light microscope. As a result, the endoneural fluid pressure increases and edema is formed within the nerve (3), which causes the neural tissue to be replaced by fibrous tissue. Gelberman and Lunborg have proposed similar pathophysiologic changes in acute nerve compression (21). Animal studies have demonstrated that the application of 20-30 mmHg pressures on peripheral nerves reduces the epineural blood flow, and is a model for low-grade peripheral nerve compression. Axonal transport is disrupted with a pressure of 30 mmHg, and the endoneural fluid pressure increases if the compression continues with this much pressure. Compression of the median nerves with a pressure of 30 mmHg in healthy volunteering humans led to mild neurophysiological changes and hand paresthesias. In patients with carpal tunnel syndrome, the mean intracarpal tunnel pressure was found to be 32 mmHg (21).

In humans, the compressed nerves are rarely examined pathologically. There have been studies on 12 median and ulnar nerves and 10 lateral femoral cutaneous nerves that were compressed to lesser extents. Histological examinations of the specimens obtained in human studies have demonstrated epineural fibrosis at the beginning of the chronic compression, followed by perineural thickening and finally endoneural fibrosis. Along with these connective tissue changes, dropping out of the large myelinated fibers localized at the periphery of fascicles is observed (32).

In a study conducted in rats about aging, reduced maximal density of the NMDA sensitive glutamate binding sites within the hippocampus was demonstrated. L-glutamate and L-aspartate are the major excitatory amino acids within the central nervous system (Watkins and Evans, 1981). The postsynaptic responses to excitatory amino acids are mediated pharmacologically by receptors with NMDA and non-NMDA subtypes. The non-NMDA

receptors take part in formation of the fast component of excitatory postsynaptic potentials, while NMDA receptors have their main roles in the modulation of plasticity and synaptic excitability via acting in long terms, as in the case of functions like learning and memory (9,16,37). Acetyl-L-carnithine is converted in the body to acyl-carnithine. It has essential physiologic roles in the mitochondrial oxidative energy metabolism (29, 34, 36). It can safely be administered orally or parenterally. Exogenous ALC administration has been shown to facilitate neurite growth in vitro (35). The positive effects of ALC on normal and pathologic aging observed in experimental and clinical studies have been attributed to cholinergic effects of the drug (22, 27). Angelucci has shown that long-term ALC treatment protects the hippocampal and pyramidal cells. The potential neuroprotective and neurotrophic effects of ALC have also been suggested in other studies (Taglialatela et al, 1991, 1992; Manfridi et al, 1992). However, there has been no report until today on the administration of this or a similar drug in compression neuropathy. It has been shown to inhibit the ammonium-induced proteolysis of MAP-2 (microtubule-associated protein), an event controlled by NMDA (13-17, 34, 37, 39).

ALC prevents the age-related reduction in p75NGFR and mRNA levels in the basal brains in old rats, but does not affect the response to stressful stimuli. The cerebellar p75NGFR and mRNA levels in old rats have been increased by ALC treatment to almost those of the younger controls (43). These results have demonstrated that the neuroprotective effect of ALC is executed through modifying the transcription of p75NGFR in central cholinergic neurons (43). There are evidences that NGF, present in peripheral and central neural system neurons, has important roles on the modulation of hypothalamo-pituitary-adrenocortical axis. ALC treatment, influencing several degenerative processes related to aging, prevents the stress-related reduction in NGF binding in the central nervous system (34, 36).

Sensorial functions are significantly affected by peripheral nerve traumas. Previous studies have demonstrated that 35% of all neurons in a dorsal root ganglion are lost even if the severed nerve is urgently operated neurosurgically. Neurotrophins such as NGF and NT-3 (neurotrophin-3) in the dorsal root ganglia of rats have been shown to reduce the sensorineural loss following peripheral nerve trauma (2, 4, 11, 12, 23, 26, 28, 30, 31, 40- 42).

ALC relieves diabetic neuropathy via improving axoplasmic transport. Unlike adrenalin, ALC does not completely affect the anterograde or both types of transport in diabetic neurons, but acts via improving the disrupted retrograde transport (Takenaka, 1994). ALC increases both the amount, and rate of retrograde particle transport in diabetic neurons by 31%, and 16%, respectively (28).

ALC acts via:

1. Increasing the NGF (nerve growth factor)-binding capacity of neurons, in turn increasing the response to neurotrophine (18).
2. Potentiates the effects of NGF.
3. Providing an acetyl group for the continuity of aerobic glycolysis with high-energy substrates, and facilitating the transport of cytosolic long-chained free fatty acids through the mitochondrial membrane (7).
4. Prevents the disruption of mitochondrial oxidative metabolism (particularly in regenerating neurons that have higher energy demands)(5, 6, 8, 13,).
5. May protect the cells with its antioxidant role from damage caused by reactive oxygen radicals.
6. Increasing motor nerve regeneration following peripheral axotomy, and sensorineural survival, which have been proven experimentally in vitro (11,18, 25).
7. Clinically, ALC has a perfect safety profile. It is being researched in diabetic neuropathy treatment, and has been shown to improve pudental nerve functions in these patients.
8. Studies on ALC administration to neuropathic patients have demonstrated significant improvements in nerve conduction velocities.
9. In patients with HIV-related peripheral neuropathy, it reduces the neuropathic pain syndromes. It provides significant regeneration in the cutaneous nerve fibres in sweat glands, dermis and epidermis.
10. In addition to its neuroprotective effects, it significantly improves the regenerative capacity of neurons following peripheral nerve traumas (39, 41).

In diabetic rats, intraperitoneal injections have been sufficient to normalize the sciatic nerve ALC levels. Both parenteral and enteral ALC treatments have been shown to increase the plasma and central nervous system levels (28).

The most important problem in conducting a study on chronic compression neuropathy is which model to choose. Though several models have been developed to study the acute compression neuropathy, chronic compression neuropathy models have not been fully idealized. As rabbit and guinea pig models are developmental models, observation of central adaptive changes renders these models irrational. In developing an experimental compression neuropathy model, application of a silicon tube has several advantages; both compression and decompression procedures can be easily performed with a silicon tube.

Elasticity of the silicon tube minimizes the peripheral nerve trauma during application, therefore preventing the development of pathologically confounding results that are actually observed in other types of nerve trauma. In compression, silicon tubes do not cause foreign material reactions, infections or interact with the nervous tissue.

We suggest that longer durations are not necessary to induce chronic compression neuropathy in rats.

The statistically significant difference we have found between the group with chronic compression neuropathy induction (group 2) and the group with ALC administration following decompression (group 4) ($p<0.008$) indicates that the right soleus muscle weights differ with ALC administration. Absence of this difference between the group 2 and the group with surgical decompression only (group 3) indicates that ALC administration has additional positive effects.

Absence of a statistically significant difference between the group with decompression only (group 3) and ALC administration without decompression (group 5) indicates that surgical decompression alone is not more important in treating the compression neuropathy than drug administration. One reason for this result can be the minor trauma that occurs during surgical decompression.

The observation of a statistically significant difference between the group with chronic compression neuropathy and the group with ALC administration without decompression (group 5) ($p<0.008$) indicates that ALC administration without decompression may prove beneficial in the treatment of compression neuropathies. As these conclusions were based on the weight of the soleus muscle that is innervated by the sciatic nerve, the action of ALC is assessed indirectly.

CONCLUSION

The implication of these results on neurosurgery practice can be that the administration of this drug following decompression of a nerve may prove revolutionary in peripheral nerve surgery. However, we suggest that more extensive studies on this area should be performed.

REFERENCES

1. Amadio PC : The Mayo Clinic and carpal tunnel syndrome. Mayo Clin Proc 67:42-8,1992
2. Bahadori MH, Al-Tiraihi T, Valojerdi MR. Sciatic nerve transection in neonatal rats induces apoptotic neuronal death in L5 dorsal root ganglion. J Neurocytol 30(2):125-30, 2001
3. Bergmark M, Kanje M, Widerberg A, Dahlin LB. Experimental nerve compression and upregulation of CPON in DRG. NeuroRapor 4;12(17):3783-6, 2001
4. Bigini P, Larini S, Pasquali C, Muzio V, Mennini T. Acetyl-L carnitine shows neuroprotective and neurotrophic activity in primary culture of rat embryo motoneurons.Neurosci Lett 6;329(3):334-8, 2002
5. Binienda ZK . Neuroprotective effects of L-carnitine in induced mitochondrial dysfunction. Ann N Y Acad Sci 993:289-95; discussion 345-9, 2003
6. Bora FW Jr, Osterman AL. Compression neuropathy. Clin Orthop 163:20-32, 1982
7. Bremer J. The role of carnitine in intracellular metabolism. J Clin Chem Clin Biohem 28(5):297-301,1990
8. Castaneda F, Kinne RK. Omental graft improves functional recovery of transected peripheral nerve. Muscle Nerve 26(4):527-32, 2002
9. Castorina M, Ambrosini AM, Giuliani A, Pacifici L, Ramacci MT, Angelucci L. A cluster analysis study of acetyl-L-carnitine effect on NMDA receptors in aging. Exp Gerontol 28(6):537-48, 1993
10. Chauhan NB, Siegel GJ. Effect of PPF and ALCAR on the induction of NGF- and p75- mRNA and on APP processing in Tg2576 brain. Neurochem Int 43(3):225-33, 2003
11. De Angelis C, Scarfo C, Falcinelli M, Perna E, Ramacci MT, Angelucci L. Age- and trauma-dependent modifications of neuromuscular junction and skeletal muscle structure in the rat. Effects of long-term treatment with Acetyl-L-Carnitine. Mech Ageing Dev 3;85(1):37-53, 1995
12. De Grandis D, Santoro L, Di Benedetto P. L-acetylcarnitine in the treatment of patients with peripheral neuropathies. Clin Drug Invest 10:317-322, 1995
13. Dhitavat S, Ortiz D, Shea TB, Rivera ER. Acetyl-L-carnitine protects against amyloid-beta neurotoxicity: roles of oxidative buffering and ATP levels. Neurochem Res 27(6):501-5, 2002
14. Dolezal V, Tucek S. Utilization of citrate, acetylcarnitine acetate, pyruvate and glucose for the synthesis of acetylcholine in rat brain slices. J Neurochem 36:1323-30, 1981
15. Evans GR. Challenges to nerve regeneration. Semin Surg Oncol 19:312-318, 2000

16. Foreman PJ, Perez-Polo JR, Angelucci L, Ramacci MT, Taglialatela G. Effects of acetyl-L-carnitine treatment and stress exposure on the nerve growth factor receptor (p75NGFR) mRNA level in the central nervous system of aged rats. Prog Neuropsychopharmacol Biol Psychiatry 19(1):117-33, 1995
17. Forloni G, Angeretti N, Smiroldo S. Neuroprotective activity of acetyl-L-carnitine: studies in vitro. J Neurosci Res 37(1):92-6, 1994
18. Formenti A, Arrigoni E, Sansone V, Arrigoni Martelli E, Mancia M. Effects of acetyl-L-carnitine on the survival of adult rat sensory neurons in primary cultures. Int J Dev Neurosci 10(3):207-9, 1992
19. Fritz IB, Marquis NR. The role of acylcarnitine esters and carnitine palmityltransferase in the transport of fatty acyl groups across mitochondrial membranes. Proc Nat Acad Sci 54:1226-33, 1965
20. Fowler TS, Ochoa J. Unmyelinated fibers in normal and compressed peripheral nerves of the baboon: A Quantitative electron microscopic study. Neuropath Applied Neurobiol 23:247-254, 1975
21. Gelberman RH, Rydevik BL, Pess GM, Szabo RM, Lundborg G. Carpal tunnel syndrome. A scientific basis for clinical care. Orthop Clin North Am 19(1):115-24, 1988
22. Ghelardini C, Galeotti N, Calvani M, Mosconi L, Nicolai R, Bartolini A. Acetyl-l-carnitine induces muscarinic antinocieption in mice and rats. Neuropharmacology 43(7):1180-7, 2002
23. Goettl VM, Neff NH, Hadjiconstantinou M. Sciatic nerve axotomy in aged rats: response of motoneurons and the effect of GM1 ganglioside treatment. Brain Res 4;968(1):44-53, 2003
24. Haldeman S, Meyer BJ: The effect of experimental constriction on the structure of the sciatic nerve. S Afr Med J 8 44(31):888-92, 1970
25. Hart AM, Wiberg M, Youle M, Terenghi G. Systemic acetyl-L-carnitine eliminates sen sory neuronal loss after peripheral axotomy: a new clinical approach in the management of peripheral nerve trauma. Exp Brain Res 145(2):182-9, 2002
26. Inano A, Sai Y, Nikaido H, Hasimoto N, Asano M, Tsuji A, Tamai I. Acetyl-L-carnitine permeability across the blood-brain barrier and involvement of carnitine transporter OCTN2. Biopharm Drug Dispos 24(8):357-65, 2003
27. Janiri L, Tempesta E. A pharmacological profile of the effects of carnitine and acetylcarnitine on the central nervous system. Int J Clin Pharm Res 111(4);295-306, 1983 .
28. Kano M, Kawakami T, Hori H, Hashimoto Y, Tao Y, Ishikawa Y, Takenaka T. Effects of ALCAR on the fast axoplasmic transport in cultured sensory neurons of streptozotocin-induced diabetic rats. Neurosci Res 33(3):207-13, 1999
29. Levine J, Panchalingam K, McClure RJ, Gershon S, Pettegrew JW. Effects of acetyl-L-carnitine and myo-inositol on high-energy phosphate and membrane phospholipid metabolism in zebra fish: a 31P-NMR-spectroscopy study. Neurochem Res 28(5):687-90, 2003
30. Ljungberg C, Novikov L, Kellerth JO, Ebendal T, Wiberg M. The neurotrophins NGF and NT-3 reduce sensory neuronal loss in adult rat after peripheral nerve lesion. Neurosci Lett 26;262(1):29-32, 1999
31. Ma J, Novikov LN, Kellerth JO, Wiberg M. Early nerve repair after injury to the postganglionic plexus: an experimental study of sensory and motor neuronal survival in adult rats. Scand J Plast Reconstr Surg Hand Surg 37(1):1-9, 2003
32. Mackinnon SE, Dellon AL, Hudson AR, Hunter DA. Chronic nerve compression-- an experimental model in the rat. Ann Plast Surg 13(2):112-20, 1984
33. Mazzio E, Yoon KJ, Soliman KF. Acetyl-L-carnitine cytoprotection against 1- methyl-4-phenylpyridinium toxicity in neuroblastoma cells. Biochem Pharmacolv 66(2):297-306, 2003
34. Mc Daniel MA, Maier SF, Einstein GO. "Brain-specific" nutrients: a memory cure? Nutrition 9 (12):957-75, 2003
35. McKay Hart A, Wiberg M, Terenghi G. Pharmacological enhancement of peripheral nerve regeneration in the rat by systemic acetyl-L-carnitine treatment. Neurosci Lett1 6;(3):181-5, 2002
36. Ori C, Freo U, Pizzolato G, Dam M. Effects of acetyl-L-carnitine on regional cerebral glucose metabolism in awake rats. Brain Res 4;951(2):330-5, 2002
37. Rao KV, Qureshi IA. Reduction in the MK-801 binding sites of the NMDA sub-type of glutamate receptor in a mouse model of congenital hyperammonemia: prevention by acetyl-L-carnitine. Neuropharmacology 38(3):383-94, 1999
38. Shu N. The effect of neurolysis on the recovery of experimentally induced entrapment neuropathy. Nippon Seikeigeka Gakkai Zasshi 69(7):517-27,1995
39. Taglialatela G, Angelucci L, Ramacci MT, Werrbach-Perez K, Jackson GR, Perez-Polo JR. Stimulation of nerve growth factor receptors in PC12 by acetyl-L-carnitine. Biochem Pharmaco l4;44(3):577-85, 1992
40. Weisl H, Osborne GV. The pathological changes in rats' nerves subject to moderate compression. J Bone Joint Surg Br 46:297-306,1964
41. Wiberg M, Ljungberg C, O'Byrne A, Brown R, Whitworth I, Liss A, et al:Primary sensory neuron survival following targeted administration of nerve growth factor to an injured nerve. Scand J Plast Reconstr Surg Hand Surg 33(4):387-92, 1999
42. Wilson AD, Hart A, Brannstrom T, Wiberg M, Terenghi G. Primary sensory neuronal rescue with systemic acetyl-L-carnitine following peripheral axotomy. A dose-response analysis. Br J Plast Surg 56(8):732-9, 2003
43. Yasui F, Matsugo S, Ishibashi M, Kajita T, Ezashi Y, Oomura Y, et al: Effects of chronic acetyl-L-carnitine treatment on brain lipid hydroperoxide level and passive avoidance learning in senescence-accelerated mice. Neurosci Lett 16;334(3):177- 80, 2002
44. Yavuzer R, Ayhan S, Latifoğlu O, Atabay K. Turnover epineural sheath tube in Primary repair of peripheral nerves. Ann Plast Surg 48 (4): 392-400, 2000