Acetyl-L-carnitine in painful peripheral neuropathy: a systematic review

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Abstract: Acetyl-L-carnitine (ALC) has shown a neuroprotective effect in patients with peripheral neuropathies of different etiologies. Preclinical studies demonstrated a central anti-nociceptive action, both in neuropathic and nociceptive pain models. The present review aims to provide the knowledge on the efficacy of ALC in patients with painful peripheral neuropathy, based on the evidence. Consistent with the PRISMA statement, authors searched PubMed, Embase and the Cochrane Database of Systematic Reviews for relevant papers, including those issued before April 2018. Two authors independently selected studies for inclusion and data extraction: only trials including patients with a diagnosis of peripheral neuropathy and involving at least 10 patients were considered for the purposes of this review. Fourteen clinical trials were revised, to provide the level of evidence for neuropathy. To assess the global efficacy of ALC in painful peripheral neuropathy, a meta-analysis of four randomized controlled trials was performed. Mean difference in pain reduction as measured on a 10-cm VAS, and 95% CIs were used for pooling continuous data from each trial. Four randomized controlled trials tested ALC in patients with neuropathy secondary to diabetes and to antiretroviral therapy for HIV. Compared to placebo, ALC produced a significant pain reduction equal to 20.2% (95% CI: 8.3%-32.1%, P<0.0001) with respect to baseline. Clinical trials also showed beneficial effects on nerve conduction parameters and nerve fiber regeneration, with a good safety profile. These data indicate that ALC provides an effective and safe treatment in patients with painful peripheral neuropathy. We recommend further studies to assess the optimal dose and duration of the therapeutic effect (also after treatment withdrawal).

Keywords: neuropathic pain, treatment, neuroprotective function, epigenetic mechanism

Introduction

An improved understanding of the pathophysiologic mechanism of neuropathic pain has led to the use of previously unexplored therapies, with encouraging results: among them, the acetyl-L-carnitine (ALC), ie the acetyl ester of L-carnitine produced by the human brain, liver and kidney, represents one of such recent therapeutic approaches. This molecule is an acetyl-group donor and plays an important role on mitochondrial energy homeostasis and detoxification. Besides strengthening the actions of Nerve Growth Factor (NGF) actions, and promoting peripheral nerve regeneration, ALC revealed a neuroprotective function in vitro and in vivo and in animal models of diabetic neuropathy. ALC has antiapoptotic effects in peripheral mononeuropathy models, an antioxidant activity and accretes acetylcholine production. Microdialysis studies have shown an increased acetylcholine release in both rat striatum and hippocampus following ALC administration.
Due to its analgesic effect, ALC has gained a growing clinical interest in different forms of chronic-pain neuropathy, not only for treatment, but also for pain prevention.\(^{14,15}\) Several experimental models of neuropathic pain documented the antinociceptive effect of ALC; such model encompassed streptozotocin- and chemotherapy-induced neuropathy, as well as the sciatic nerve chronic constriction injury.\(^{6,16}\) Moreover, ALC provides a significant antinociceptive effect even after the development of neuropathic pain. These analgesic properties result from different mechanisms. ALC is the only drug whose analgesic effect is due to an epigenetic mechanism, based on the acetylation of p65/RelA, a transcription factor belonging to the NFkB family. Acetylation of p65/RelA leads to a strengthened expression of type-2 metabotropic glutamate (mGlu2) receptors in the dorsal root ganglia and dorsal horns of the spinal cord, thus reducing the glutamate release from primary afferent sensory fibers.\(^{17,18}\) The analgesic effect induced by ALC persisted for at least 14 days after drug interruption. A long-term analgesic effect was also observed in mice after chronic constriction injury of the sciatic nerve.\(^{17}\)

The effect on pain of ALC is also modulated by nicotinic and muscarinic antagonists, as shown in a number of animal studies, thus suggesting the role of the cholinergic pathway in the antinociceptive activity of this drug.\(^{19,20}\) ALC may raise the uptake of acetyl-CoA into the mitochondria and, due to its similarity in structure to acetylcholine, it may also produce cholinomimetic effects.\(^{21}\)

This systematic review and meta-analysis aim to provide the actual knowledge, based on the evidence, of ALC efficacy compared to placebo in the treatment of neuropathic pain in patients with peripheral neuropathy.

### Materials and methods

#### Search process

We searched PubMed, Embase and the Cochrane Database of Systematic Reviews for relevant papers, considering publications issued before April 2018. The following search terms were used: “acetyl-L-carnitine”, “neuropathic pain”, and “neuropathy”. Full-length, original communications were included, limiting the search to English-language publications. The review process was carried out by two reviewers: only publications independently approved by the two authors were taken into account (Figure 1). The following inclusion criteria were considered: trials including patients with a diagnosis of neuropathic pain related to peripheral neuropathy, and a minimum sample size of 10 patients. A revision of the selected clinical trials was carried out, to provide the level of evidence, according to the guidelines for clinical practice recommendations of the American Academy of

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**Figure 1** Flowchart of the search process.

**Abbreviation:** RCTs, randomized controlled trial.
Neurology (AAN). A meta-analysis of randomized control trials (RCTs), testing the effect of ALC in patients with painful peripheral neuropathy was also performed. For the purposes of the meta-analysis, we considered only the RCTs with a homogeneous measure of the ALC effect on pain, compared to placebo. The risk of bias of the included RCTs was evaluated independently by two reviewers, in accordance with the guidelines provided by the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0.

Statistical analysis
We pooled the mean reduction of pain, compared to placebo, as measured on a 10-cm VAS. The pooled effect of each study was described in terms of mean difference (MD) with 95% CIs. Heterogeneity among studies was assessed by analysing the relevant forest plots; the statistical analysis consisted of a $\chi^2$ test of homogeneity and the evaluation of the inconsistency index $I^2$. An inconsistency index $I^2 > 50\%$ indicated a statistically significant among-study heterogeneity: in this case, studies were pooled using random-effects model. A subgroup analysis, based on the cause of neuropathy (diabetes and antiretroviral toxic neuropathy), was carried out; heterogeneity between groups was also evaluated. Forest plots of the individual studies and the pooled average were also computed, with the aid of GraphPad Prism 7.0.

Results
Clinical trials overview
Out of 44 full-text articles, the search process led to the selection of fourteen clinical trials, whose characteristics are summarized in Table 1. Such trials encompassed both RCTs and open-label studies. To each of these studies we assigned a classification level according to the AAN method. Among them, only four RCTs were considered for the meta-analysis.

Acetyl-L-carnitine in diabetic neuropathy
Three Class II RCTs compared ALC versus placebo in a total of 1590 patients with diabetic peripheral neuropathy. The treatment efficacy on pain was evaluated using a VAS.

Sima and colleagues carried out two RCTs with the same design. ALC was administered at two doses (500 or 1,000 mg) three times a day (t.i.d.) for 1 year. Patients treated with 1,000 mg ALC t.i.d. showed significant improvements at both 26 and 52 weeks. Type 2 diabetes, adequate drug compliance, and HbA1c > 8.5% were associated to the greatest benefit in pain reduction. Pain relief was linked to improvements in clinical symptom scores and morphometric parameters of sural nerve biopsy, ie the increased fiber numbers and clusters of regenerating fibers. No significant differences in nerve conduction study data and in the incidence of adverse events between the two groups of patients were observed.

In the RCT of De Grandis and colleagues, 1,000 mg/day of ALC were administered intramuscularly for 10 days; the dosage was then raised to 2,000 mg/day, administered orally, until the end of the study (355 days). After 12 months of treatment, a significant reduction in the mean VAS scores for pain was observed in patients treated with ALC, compared with the placebo group. A significant improvement in nerve conduction study parameters was also found in treated patients. No serious adverse events were reported.

A multicenter, double-blind RCT assessed the efficacy and safety of ALC in diabetic peripheral neuropathy compared with methylcobalamin. The study encompassed 232 patients, randomized to receive oral ALC 500 mg t.i.d. or methylcobalamin 0.5 mg t.i.d. for 24 weeks. At the end of the treatment period, patients from both groups showed significant reductions in both the neuropathy symptom score and neuropathy disability score, with no meaningful difference between the two groups. Neurophysiological parameters were also improved in both groups.

Acetyl-L-carnitine in antiretroviral toxic neuropathy
One Class II RCT was conducted in patients with antiretroviral toxic neuropathy. This is a double-blind placebo-controlled study testing the safety and efficacy of ALC compared to placebo in the treatment of pain in HIV-positive patients with distal symmetric polyneuropathy related to antiretroviral drugs. Ninety patients were included in the trial, randomized to receive ALC 1,000 mg/day (500 mg intramuscularly twice daily) during the 14-day double-blind phase. During the 42 days of open-treatment follow-up phase, ALC was administered in the form of 1,000 mg oral sachets twice a day. The treatment efficacy on pain was evaluated using VAS, Total Symptom Score (TSS), Clinical Global Impression of Change, McGill Pain Questionnaire (MPQ), and the need
| Author/year                                      | Study type          | Neuropathy                  | Sample Size | Study duration (months) | ALC daily dose (mg) | Outcome Measures                                      | Findings                                                                                          | Class |
|-----------------------------------------------|---------------------|-----------------------------|-------------|-------------------------|---------------------|-------------------------------------------------------|---------------------------------------------------------------------------------------------------|-------|
| Sima et al United Stated, Canadian study (UC), 2005 | RCT                 | Diabetic peripheral neuropathy | 1257        | 13                      | 500 or 1000         | VAS scale, NCS                                        | VAS reduction: $-25.53 \pm 28.75 \text{ mm}$<br>VAS reduction: $-21.75 \pm 34.58 \text{ mm}$   | II    |
| De Grandis et al, 2002                         | RCT                 | Diabetic peripheral neuropathy | 333         | 12                      | 2000                | VAS scale, NCS                                        | VAS reduction: $-39\%$ compared to baseline<br>NCS improvement: $+5.7 \text{ m/sec}$ compared to baseline | II    |
| Sheyu et al, 2016                              | RCT                 | Diabetic peripheral neuropathy | 232         | 6                       | 500                 | NCS and neuropathy symptom score                      | NCS improvement: $5.03 \pm 0.78 \text{ m/sec}$<br>NSS improvement: $4.01 \pm 3.25$           | II    |
| Youle et al, 2007                              | RCT                 | Antiretroviral toxic neuropathy | 90          | 0.5                     | 1000                | VAS scale                                             | VAS reduction: $-0.89/+/0.75 \text{ mm}$                                               | II    |
| Hart et al, 2004                               | Open-label uncontrolled trial | Antiretroviral toxic neuropathy | 21          | 33                      | 3000                | Skin innervation                                      | Epidermal, dermal and sweat gland innervation reached 92%, 80% and 69%, respectively, after 6-month treatment | IV    |
| Valcour et al, 2009                            | Open-label uncontrolled study | Antiretroviral toxic neuropathy | 21          | 6                       | 3000                | GPIS, skin innervation, mtDNA copies/cell             | GPIS improvement: $-0.079$ compared to baseline<br>No changes in skin innervation and mtDNA copies/cell | IV    |
| Osio et al, 2006                               | Open-label uncontrolled study | Antiretroviral toxic neuropathy | 20          | 1                       | 2000                | Short-form McGill Pain Questionnaire                 | Pain reduction: $-21\%$ compared to baseline                                               | IV    |
| Scarpini et al, 1997                           | Open-label uncontrolled study | Antiretroviral toxic neuropathy | 16          | 0.75                    | 500 or 1000        | Huskisson's analogic scale                            | Pain reduction in 62.5% of subjects                                                        | IV    |
| Sun et al, 2016                                | RCT                 | Chemotherapy induced neuropathy | 239         | 2                       | 3000                | Peripheral neuropathy grade (NCI-CTC), NCS            | Reduced neurotoxicity: 50.5% of patients<br>NCV improvement: 60.7% of patients | II    |

(Continued)
| Author/year          | Study type                  | Neuropathy                      | Sample Size | Study duration (months) | ALC daily dose (mg) | Outcome Measures            | Findings                                                                 | Class |
|---------------------|-----------------------------|--------------------------------|-------------|-------------------------|---------------------|-----------------------------|----------------------------------------------------------------------------|-------|
| Maestri et al, 2005 | Open-label uncontrolled study | Chemotherapy induced neuropathy | 27          | 0.25                    | 1000                | Peripheral neuropathy grade (WHO) | At least one WHO grade improvement in 73% of patients                      | IV    |
| Bianchi et al, 2005 | Open-label uncontrolled study | Chemotherapy induced neuropathy | 25          | 2                       | 3000                | Total neuropathy score      | TNS improvement: 92% of patients                                         | IV    |
| Cruccu et al, 2018  | Open-label uncontrolled study | Carpal tunnel syndrome         | 82          | 4                       | 1000                | NCS, BCTQ, NPSI             | SCV changed from 34.7±5.6 m/s at t0 to 36.7±6.6 m/s at t120
Symptom and functional BCTQ decrease: −39% and 18% compared to baseline
NPSI decrease: −38% to −56% compared to baseline                      | IV    |
| De Grandis et al, 1995 | RCT                         | Peripheral neuropathy of different etiologies | 426         | 1                       | 2000                | NCS                         | NCV improvement in patients with mononeuropathies and in sensory nerve neuropathies, compared to placebo | II    |

**Abbreviations:** ALC, acetyl-L-carnitine; RCT, randomized controlled trial; NCS, nerve conduction study; NSS, neuropathy symptom score; NCI-CTC, National Cancer Institute-Common Toxicity Criteria; GPI, gracely pain intensity scale; NCV, nerve conduction velocity; TNS, total neuropathy score; SCV, sensory conduction velocity; BCTQ, Boston Carpal tunnel questionnaire; NPSI, neuropathic pain symptom inventory.
for rescue analgesics. For the efficacy-evaluable population, the group of patients treated with ALC showed a significantly greater reduction in pain, compared to the placebo group. During the open-label phase, VAS, TSS and MPQ revealed a pain relief. Intramuscular and oral treatments were generally safe and well tolerated; the viral load, CD4/CD8 ratio and CD4 and CD8 counts remained stable, thus revealing that ALC treatment was not associated with any progression of HIV infection.

Several open-label studies including patients with antiretroviral toxic neuropathy have been carried out. One study, involving 21 HIV-positive patients with established neuropathy, assessed the effect of oral ALC (1,500 mg twice daily) on dermal and intraepidermal innervation. After a 6-month treatment, the mean immunostaining area for small sensory fiber increased in all fiber types, including sympathetic fibers: the epidermal, dermal and sweat gland innervation reached 92%, 80% and 69%, respectively compared with the control group. Neuropathic pain grade improved in 76% of patients, whereas it remained unchanged in 19%. No association of ALC treatment with any progression of HIV infection was observed. An open-label, single-arm pilot study involving 21 patients, evaluated the effect of 3,000 mg ALC daily on the intra-epidermal nerve fiber (IENF) density and mitochondrial DNA (mtDNA) copies/cell. Whereas IENF density and mtDNA copies/cell did not change after therapy, improvements in neuropathic pain, paresthesias, and symptoms of numbness were observed. An open-label study, involving 20 subjects with painful antiretroviral toxic neuropathy, tested the efficacy of oral ALC at a dose of 2,000 mg/day for a 4-week period. Mean pain intensity score, evaluated using the modified short-form MPQ, was significantly lowered during the study, whereas electrophysiological parameters did not show significant changes. Other two open label studies, totaling 26 patients, revealed a positive effect of ALC in reducing neuropathic pain intensity.

### Acetyl-L-carnitine in chemotherapy induced neuropathy

Clinical studies tested the neuroprotective effect of ALC in chemotherapy-induced neuropathy but no data about neuropathic pain reduction are available.

A prospective, double-blind, Class II RCT study totaling 239 patients with chemotherapy-induced peripheral neuropathy tested the effect of oral administration of
This study considered as primary endpoint the improvement of peripheral neuropathy by at least one grade according to the National Cancer Institute Common Toxicity Criteria version 3.0. Patients’ conditions were assessed at week 4, 8, and 12 after enrollment. At week 8, 51.6% patients treated with ALC met the primary endpoint, compared with 23.1% of patients in the placebo group. Secondary endpoints, such as sural nerve conduction velocity and the Karnofsky physical score showed also a significant improvement in patients treated with ALC, compared to the placebo group. No significant difference in the incidence of adverse events between the two groups was observed.

Two open label studies tested the effect of ALC in patients with neuropathy induced by paclitaxel and cisplatin.35,36 Maestri and colleagues tested the effect of ALC 1 g/day i.v. infusion over 1–2 h for at least 10 days in 27 patients; the peripheral neuropathy improved in 73% of them. Bianchi and colleagues tested oral ALC (1 g t.i.d.) for 8 weeks in 25 patients. The total neuropathy score, including neurophysiological measures, improved in 92% of them.

Three studies totaling 578 patients with cancer investigated the effect of ALC in preventing chemotherapy-induced neuropathy, but no positive effect was detected.37–39

**Acetyl-L-carnitine in patients with carpal tunnel syndrome**

Neuropathic pain is a common symptom in patients with Carpal tunnel syndrome (CTS).40

A recent multicenter, examiner-blinded, clinical and neurophysiological study assessed the efficacy of ALC on neuroprotection, pain, and function in CTS.15 The study included eighty-two patients with CTS of mild-to-moderate severity. Patients’ conditions were assessed at baseline and after 10, 60, and 120 days of treatment. After a first 10-day period of intramuscular injections, ALC 500 mg b.i.d., patients received an oral treatment consisting of one tablet of ALC 500 mg b.i.d., for 110 days. Each patient underwent a median nerve conduction study, the Boston Carpal Tunnel Questionnaire (BCTQ) and the Neuropathic Pain Symptom Inventory (NPSI). The BCTQ score was significantly improved, especially in the symptom component. Squeezing, pressure pain and pain evoked by pressure, were significantly lowered. These symptom improvements were detected after the first 10 days of intramuscular treatment and persisted throughout the 4-month treatment period. All sensory neurophysiological measures significantly improved.

**Meta-analysis of RCTs in diabetic and HIV-related painful peripheral neuropathy**

Four RCTs tested the effect of ALC in comparison with placebo in patients with diabetic and antiretroviral toxic neuropathy (Table 2). A random-effects model was used for the analysis, given that the heterogeneity indexes approached the statistical significance ($\tau^2=88.58; \chi^2=8.06, df=3, P=0.045; I^2=62.8\%$). Compared to placebo, ALC produced a pain reduction equal to 20.2% (95% CI: 8.3–32.1%, $P<0.0001$) with respect to baseline. The forest plot, displaying the results from individual studies as well as the pooled effect with the relevant CI, is reported in Figure 2. A subgroup analysis considering only the three studies conducted in diabetic peripheral neuropathy was carried out (Table 3). Even in this case, a random-effects model was used ($\tau^2=50.28; \chi^2=3.40, df=2, P=0.18; I^2=41.2\%$). With respect to baseline, the mean difference in pain
reduction was equal to 24.6% (95% CI: 12.4–36.8%, \( P<0.0001 \)): these results match those reported by Li and colleagues.\(^\text{14}\) The test addressing the differences between the diabetic subgroup versus the HIV therapy-related study revealed a meaningful heterogeneity (\( \chi^2=2.31, \ df=1, \ P=0.13; \ I^2=56.7\% \)). Table 4 provides details on the risk of bias assessment for each study. In general, reporting of randomization methods and treatment allocation was clearly stated in De Grandis and colleagues\(^\text{25}\) and in Youle and colleagues\(^\text{28}\) whereas it was uncertain in Sima and colleagues\(^\text{24}\) therefore associated with an uncertain bias risk. Allocation concealment and blinding were addressed in De Grandis and colleagues.\(^\text{25}\)

### Effects of ALC on nerve function

Controlled trials in large cohorts of patients with peripheral neuropathy of different etiologies tested the effect of ALC on neurophysiological measures. In the double-blind RCT of De Grandis and colleagues, involving 333 patients with diabetic neuropathy, the mean nerve conduction velocity and amplitude significantly improved, in comparison with placebo.\(^\text{25}\) In two randomized placebo-controlled trials, totaling 1257 patients with diabetic neuropathy, Sima and colleagues failed to find any significant electrophysiological change in patients treated with 500 or 1,000 mg ALC, although a significant improvement in the vibration perception threshold was reported.\(^\text{24}\) A short-term, double-blind clinical study involving 426 patients with peripheral neuropathy of different etiologies, showed statistically meaningful differences between the ALC and placebo groups in terms of mean conduction velocity improvement.\(^\text{41}\)

A double-blind, randomized placebo-controlled study, totaling 239 patients with chemotherapy-induced peripheral neuropathy, reported a meaningful increase of sural nerve conduction velocity after ALC treatment.\(^\text{34}\)

The neuroprotective effect of ALC in CTS was tested in a multicenter, examiner-blinded, clinical and neurophysiological study totaling 82 patients. The primary outcome, ie the sensory conduction velocity of the median nerve, significantly improved after 4 months of treatment. Such an improvement, detected after the first 60 days of treatment, persisted throughout the treatment period, lasting 4 months. The sensory action potentials amplitude of the median nerve increased from baseline to the end of the study.\(^\text{15}\) In addition, both the symptom and functional BCTQ scores significantly decreased.
Several works, describing different etiologies, were included. The length of follow-up was relatively short- and the long-term impact of ALC is unknown. According to recent systematic review and meta-analysis the effect of ALC on VAS in patients with painful neuropathy was similar for different administration routes (intramuscular and oral sequential administration and oral administration only).14

A recent multicenter, examiner-blinded, clinical and neurophysiological study assessed the effects of ALC in patients with mild to moderate CTS.15 By means of its neuroprotective action and central anti-nociceptive properties, ALC provided a significant pain reduction, as measured by NPSI questionnaire. More in detail, squeezing pain, pressure pain and pain evoked

Discussion

According to preclinical and clinical studies, ALC can be considered both an etiological and symptomatic treatment in patients with peripheral neuropathy, with a good safety profile. ALC operates via several mechanisms, inducing regeneration of injured nerve fibers, reducing oxidative stress, promoting DNA synthesis in mitochondria, and increasing NGF concentrations in neurons, thus promoting neurite extension.3,7 A lack of carnitine reduces energy synthesis by impairing fatty acid degradation: this condition was reported in association with diabetes and its complications.42,43 A cross-sectional study in HIV patients treated with antiretroviral nucleoside analogs showed that in patients with axonal peripheral neuropathy ALC levels are significantly lower compared to the control groups.44 Under specific conditions, the demand for ALC may exceed the capacity to synthesize this essential micronutrient.25 In addition, ALC production may be impaired by genetic defects.

ALC showed analgesic properties, by relieving acute and in chronic pain. Several clinical studies reported an improvement in symptoms after ALC supplementation in patients with peripheral neuropathy of different etiologies.31,36,45 Several works, describing different neuropathic pain models, confirmed the antinociceptive effect of ALC. Such an effect results from different mechanisms, including the activation of muscarinic cholinergic receptors, and the increased expression of mGlu2 receptors in dorsal root ganglia neurons, by means of an acetylation mechanism involving transcription factors of the nuclear factor (NF)-kappaB family.17,20 Noteworthy, the analgesic effect of ALC exceeds by several days or weeks the end of treatment, in models of chronic inflammatory and neuropathic pain. This enforces the role of ALC as an analgesic drug and supports the role of the epigenetic mechanisms in the treatment of chronic pain.17

For the first, a systematic review of literature was carried out, by providing a classification for the available clinical trials, and the level of evidence for: diabetic neuropathy, antiretroviral toxic neuropathy, chemotherapy induced neuropathy and CTS.

According to the AAN classification, ALC is probably effective in diabetic neuropathy. The presence of a single Class II trial for each type confirm the possible effect of ALC in both antiretroviral toxic neuropathy and in chemotherapy induced neuropathy. No RCT was performed in patients with CTS, but results obtained from a recent multicenter, examiner-blinded, clinical and neurophysiological study are promising.

Pooled results from the present meta-analysis, including four RCTs in patients with diabetic and antiretroviral toxic neuropathy, showed the efficacy of ALC compared to placebo in reducing pain intensity. In the subgroup analysis, patients with diabetic neuropathy reported greater pain reduction compared to patients with HIV-related peripheral neuropathy. Moderate heterogeneity, as measured by the $I^2$ index, was observed among the RCTs; no significant heterogeneity was found in studies belonging to the diabetes subgroup. This meta-analysis has some limitations: only four RCTs with small or moderate sized were included. The length of follow-up was relatively short- and the long-term impact of ALC is unknown.

| Methodological items                              | De Grandis, 2002 | Sima UC, 2005 | Sima UCE, 2005 | Youle, 2007 |
|--------------------------------------------------|------------------|--------------|---------------|-------------|
| Random sequence generation                       | Low risk         | Uncertain risk| Uncertain risk| Low risk    |
| Allocation concealment                           | Low risk         | Uncertain risk| Uncertain risk| Uncertain risk|
| Blinding of participants and personnel           | Low risk         | Uncertain risk| Uncertain risk| Uncertain risk|
| Blinding of outcome assessment                   | Low risk         | Uncertain risk| Uncertain risk| Uncertain risk|
| Incomplete outcome data                          | Uncertain        | Low risk     | Low risk      | Low risk    |
| Selective reporting                              | Low risk         |              |               |             |
| Other bias                                       |                  |              |               |             |

Table 4 Bias assessment
by pressure, were significantly lowered. Pain reduction was detected after the first 10 days of intramuscular treatment. The intramuscular administration could allow reaching high concentrations of ALC in the central nervous system, an essential condition for activating and boosting the epigenetic mechanisms underlying the analgesic action of the drug. However, once the epigenetic mechanism has been triggered, no more differences between intramuscular and oral administration are expected.

Future RCT trials in patients with painful peripheral neuropathy of different etiology are needed.

Conclusion
ALC is an effective and safe treatment in painful peripheral neuropathy, especially in diabetic patients. Future studies aiming to assess the duration of the therapeutic efficacy and the optimal dose in larger populations, possibly with longer follow-up periods, are required.

The pain reduction induced by ALC may be mediated by both a neuroprotective and a central anti-nociceptive mechanism. Future studies should investigate the role of the two mechanisms.

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References
1. Bremer J. The role of carnitine in intracellular metabolism. J Clin Chem Clin Biochem. 1990;28(5):297–301.
2. Colucci WJ, Grandour RD. Carnitine acyltransferase: a review of its biology, enzymology and bioorganic chemistry. Bioorg Chem. 1988;16:307–334. doi:10.1016/0006-2068(88)90018-1
3. Manfridi A, Forloni GL, Arrigoni-Martelli E, Mancia M. Culture of dorsal root ganglion neurons from aged rats: effects of acetyl-L-carnitine and NGF. Int J Dev Neurosci. 1992;10(4):321–329. doi:10.1016/0736-5748(92)90021-Q
4. Taglialetela G, Angelucci L, Ramacci MT, Werrbach-Perez K, Jackson GR, Perez-Polo JR. Acetyl-L-carnitine enhances the response of PC12 cells to nerve growth factor. Brain Res Dev Brain Res. 1991;59 (2):221–230.
5. Angelucci L, Ramacci MT, Taglialetela G, et al. Nerve growth factor binding in aged rat central nervous system: effect of acetyl-L-carnitine. J Neurosci Res. 1988;20(4):491–496. doi:10.1002/jnr.490200413
6. Fernandez E, Pallini R, Gangitano C, et al. Effects of L-carnitine, L-acetyl carnitine and gangliosides on the regeneration of the transected sciatic nerve in rats. Neurol Res. 1989;11(1):57–62. doi:10.1080/01616412.1989.11739863
7. Hart AM, Wiberg M, Terenghi G. Pharmacological enhancement of peripheral nerve regeneration in the rat by systemic acetyl-L-carnitine treatment. Neurosci Lett. 2002;334(3):181–185. doi:10.1016/S0304-3904(02)00982-5
8. Virmani MA, Bisseli R, Spadoni A, et al. Protective actions of L-carnitine and acetyl-L-carnitine on the neurotoxicity evoked by mitochondrial uncoupling or inhibitors. Pharmacol Res. 1995;32(6):383–389. doi:10.1016/S1043-6618(95)80444-1
9. Hart AM, Wiberg M, Youle M, Terenghi G. Systemic acetyl-L-carnitine eliminates sensory neuronal loss after peripheral axotomy: a new clinical approach in the management of peripheral nerve trauma. Exp Brain Res. 2002;145(2):182–189. doi:10.1007/s00221-002-1100-2
10. Sima AA, Ristic H, Merry A, et al. Primary preventive and secondary interventionary effects of acetyl-L-carnitine on diabetic neuropathy in the bio-breeding Worcester rat. J Clin Invest. 1996;97(8):1900–1907. doi:10.1172/JCI118621
11. Di Cesare Mannelli L, Ghelardini C, Calvani M, et al. Protective effect of acetyl-L-carnitine on the apoptotic pathway of peripheral neuropathy. Eur J Neurosci. 2007;26(4):820–827. doi:10.1111/j.1460-9568.2007.05698.x
12. Mansour HH. Protective role of carnitine ester against radiation induced oxidative stress in rats. Pharmacol Res. 2006;54(3):165–171. doi:10.1016/j.phrs.2006.04.003
13. Imperato A, Ramacci MT, Angelucci L. Acetyl-L-carnitine enhances acetylcholine release in the striatum and hippocampus of awake freely moving rats. Neurosci Lett. 1989;107(1–3):251–255. doi:10.1016/0304-3940(89)90826-4
14. Li S, Li Q, Li Y, et al. Acetyl-L-carnitine in the treatment of peripheral neuropathic pain: a systematic review and meta-analysis of randomized controlled trials. PLoS One. 2015;10(3):e0119479. doi:10.1371/journal.pone.0119479
15. Cruccu G, Di Stefano G, Fattapposta F, et al. L-Acetyl-carnitine in patients with carpal tunnel syndrome: effects on nerve protection, hand function and pain. CNS Drugs. 2018;32(3):303. doi:10.1007/s40263-018-0493-9
16. Chiocchio S, Copani A, Gereau RW 4th, Nicoletti F. Acetyl-L-carnitine in neuropathic pain: experimental data. Exp. 2017;13:1744806917697009. doi:10.1177/1744806917697009
17. Notartomaso S, Mascio G, Bernabucci M, et al. Analgesia induced by the epigenetic drug, L-acetylcarnitine, outlasts the end of treatment in mouse models of chronic inflammatory and neuropathic pain. Mol Pain. 2017;13:1744806917697009. doi:10.1177/1744806917697009
18. Truini A, Piroso P, Pasquale E, et al. N-acetyl-cysteine, a drug that enhances the endogenous activation of group-II metabotropic glutamate receptors, inhibits nociceptive transmission in humans. Mol Pain. 2015;11:14. doi:10.1186/s12990-015-0000-2
19. Di Cesare Mannelli L, Ghelardini C, Calvani M, et al. Neuroprotective effects of acetyl-L-carnitine on neuropathic pain and apoptosis: a role for the nicotinic receptor. J Neurosci Res. 2009;87(1):200–207. doi:10.1002/jnr.21815
20. Bartolini A, Di Cesare Mannelli L, Ghelardini C. Analgesic and antinociceptive drugs acting through central cholinergic mechanisms. Recent Pat CNS Drug Discov. 2011;6(2):119–140. doi:10.2174/15748891179533901
21. Pessoa BL, Escudeiro G, Nascimento OJ. Emerging treatments for neuropathic pain. Curr Pain Headache Rep. 2015;19(12):56. doi:10.1007/s11916-015-0530-z
22. American Academy of Neurology. Edition Clinical Practice Guideline Process Manual. 2017. Available from: https://www.aan.com/sites/assets/home-page/policy-and-guidelines/guidelines/about-guidelines/1?guidelinestoproman_pg.pdf. Accessed 21 March, 2019.

23. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. Res Synth Methods. 2010;1(2):97–111. doi:10.1002/jrsm.12

24. Sima AA, Calvani M, Mehra M, Amato A. Acetyl-L-Carnitine Study Group. Acetyl-L-carnitine improves pain, nerve regeneration, and vibratory perception in patients with chronic diabetic neuropathy: an analysis of two randomized placebo-controlled trials. Diabetes Care. 2005;28(1):89–94.

25. De Grandis D, Minardi C. Acetyl-L-carnitine (levacarnicaine) in the treatment of diabetic neuropathy. A long-term, randomised, double-blind, placebo-controlled study. Drugs R D. 2002;3(4):223–231.

26. Li S, Chen X, Li Q, et al. Effects of acetyl-L-carnitine and methylcobalamin for diabetic peripheral neuropathy: A multicenter, randomized, double-blind, controlled trial. J Diabetes Investig. 2016;7(5):777–785. doi:10.1111/jdi.12493

27. Phillips TJ, Cherry CL, Cox S, Marshall SJ, Rice AS. Pharmacological treatment of painful HIV-associated sensory neuropathy: a systematic review and meta-analysis of randomised controlled trials. PLoS One. 2010;5(12):e14433. doi:10.1371/journal.pone.0014433

28. Youle M, Osio M, ALCAR Study Group. A double-blind, parallel-group, placebo-controlled, multicentre study of acetyl L-carnitine in the symptomatic treatment of antiretroviral toxic neuropathy in patients with HIV-1 infection. HIV Med. 2007;8(4):241–250. doi:10.1111/j.1468-1293.2007.00467.x

29. Hart AM, Wilson AD, Montovani C, et al. Acetyl L-carnitine: a pathogenesis based treatment for HIV-associated antiretroviral toxic neuropathy. AIDS. 2004;18(11):1549–1560.

30. Valcour V, Yeh TM, Bartt R, et al. Acetyl-L-carnitine and nucleoside reverse transcriptase inhibitor-associated neuropathy in HIV infection. HIV Med. 2009;10(2):103–110. doi:10.1111/j.1468-1293.2008.00658.x

31. Osio M, Muscia F, Zapimini L, et al. Acetyl-L-carnitine in the treatment of painful antiretroviral toxic neuropathy in human immunodeficiency virus patients: an open label study. J Peripher Nerv Syst. 2006;11(1):72–76. doi:10.1111/j.1085-9489.2006.00066.x

32. Scarinci E, Sacilotto G, Baron P, et al. Effect of acetyl-L-carnitine in the treatment of painful peripheral neuropathies in HIV+ patients. J Peripher Nerv Syst. 1997;2(3):250–252.

33. Herzmann C, Johnson MA, Youle M. Long-term effect of acetyl-L-carnitine for antiretroviral toxic neuropathy. HIV Clin Trials. 2005;6(6):344–350. doi:10.1310/CHIN-YBNU-G3CU-JBXR

34. Sun Y, Shu Y, Liu B, et al. A prospective study to evaluate the efficacy and safety of oral acetyl-L-carnitine for the treatment of chemotheraphy-induced peripheral neuropathy. Exp Ther Med. 2016;12(6):4017–4024. doi:10.3892/etm.2016.3871

35. Maestri A, De Pasquale Cerrati A, Cundari S, Zanna C, Cortesi E, Cinò L. A pilot study on the effect of acetyl-L-carnitine in paclitaxel- and cisplatin-induced peripheral neuropathy. Tumori. 2005;91(2):135–138.

36. Bianchi G, Vitali G, Caraceni A, et al. Symptomatic and neurophysiological responses of paclitaxel- or cisplatin-induced neuropathy to oral acetyl-L-carnitine. Eur J Cancer. 2005;41(12):1746–1750. doi:10.1016/j.ejca.2005.04.028

37. Hershman DL, Unger JM, Crew KD, et al. Randomized placebo-controlled trial of acetyl-L-carnitine for prevention of taxane-induced neuropathy during adjuvant breast cancer therapy. J Clin Oncol. 2013;31(20):2627–2633. doi:10.1200/JCO.2012.44.8738

38. Campone M, Berton-Rigaud D, Joly-Lobbledez F, et al. A double-blind, randomized phase II study to evaluate the safety and efficacy of acetyl-L-carnitine in the prevention of sugopilone-induced peripheral neuropathy. Oncologist. 2013;18(11):1190–1191. doi:10.1634/theoncologist.2013-0061

39. Callander N, Markovina S, Eickhoff J, et al. Acetyl-L-carnitine (ALCAR) for the prevention of chemotherapy-induced peripheral neuropathy in patients with relapsed or refractory multiple myeloma treated with bortezomib, doxorubicin and low-dose dexamethasone: a study from the wisconsin oncology network. Cancer Chemother Pharmacol. 2014;74(4):875–882.

40. Truini A, Padua L, Biasiotta A, et al. Differential involvement of A-delta and A-beta fibres in neuropathic pain related to carpal tunnel syndrome. Pain. 2009;145(1–2):105–109. doi:10.1016/j.pain.2009.05.023

41. De Grandis D, Santoro L, Di Benedetto P. L-acetylcarnitine in the treatment of patients with peripheral neuropathies: a short term, double-blind clinical study of 426 patients. Clin Drug Invest. 1995;10(6):317–322. doi:10.2165/00044011-199510060-00001

42. Fritz IB. Action of carnitine on long chain fatty acid oxidation by liver. Am J Physiol. 1959;197:297–304. doi:10.1152/ajplegacy.1959.197.1.217

43. Tamamogullari N, Sili̇g I, Çağdaşioğlu S, Atalay A. Carnitine deficiency and its complications. J Diabetes Complications. 1999;13(5–6):251–253.

44. Famularo G, Moretti S, Marcellini S, et al. Acetyl-carnitine deficiency in AIDS patients with neurotoxicity on treatment with antiretroviral nucleoside analogues. AIDS. 1997;11(2):185–190.

45. Hur J, Sullivan KA, Callaghan BC, Pop-Busui R, Feldman EL. Identification of factors associated with sural nerve regeneration and degeneration in diabetic neuropathy. Diabetes Care. 2013;36(12):4043–4049. doi:10.2337/dc12-2530