Acetyl-L-Carnitine Improves Pain, Nerve Regeneration, and Vibratory Perception in Patients With Chronic Diabetic Neuropathy

An analysis of two randomized placebo-controlled trials

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OBJECTIVE — We evaluated frozen databases from two 52-week randomized placebo-controlled clinical diabetic neuropathy trials testing two doses of acetyl-L-carnitine (ALC): 500 and 1,000 mg/day t.i.d.

RESEARCH DESIGN AND METHODS — Intention-to-treat patients amounted to 1,257 or 93% of enrolled patients. Efficacy end points were sural nerve morphometry, nerve conduction velocities, vibration perception thresholds, clinical symptom scores, and a visual analogue scale for most bothersome symptom, most notably pain. The two studies were evaluated separately and combined.

RESULTS — Data showed significant improvements in sural nerve fiber numbers and regenerating nerve fiber clusters. Nerve conduction velocities and amplitudes did not improve, whereas vibration perception improved in both studies. Pain as the most bothersome symptom showed significant improvement in one study and in the combined cohort taking 1,000 mg ALC.

CONCLUSIONS — These studies demonstrate that ALC treatment is efficacious in alleviating symptoms, particularly pain, and improves nerve fiber regeneration and vibration perception in patients with established diabetic neuropathy.

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Diabetic polyneuropathy (DPN) is the most common late complication of diabetes (1) and is commonly associated with neuropathic pain. DPN shows a dynamic natural history with early reversible metabolic abnormalities, which become progressively superimposed by less reversible structural lesions and functional deficits (2).

Several clinical diabetic neuropathy trials have been undertaken in the past (rev. in 3,4). Most notably, numerous aldose reductase inhibitor (ARI) trials have been conducted with disappointing results (5–8). Because of adverse drug effects, several ARI developments were abandoned (4,9). Multicenter trials with α-lipoic acid have shown small improvements in nerve conduction velocities, but no effects on neuropathy disability scores (4,10).

Acetyl-L-carnitine (ALC) is deficient in diabetes (11,12). In preclinical studies, substitution with ALC corrects perturbations of neural Na^+/K^+-ATPase, myoinositol, nitric oxide (NO), prostaglandins, and lipid peroxidation, all of which play important early pathogenetic roles in DPN (13–16). Long-term prevention and intervention studies in the diabetic rat have revealed preventional and therapeutic effects on peripheral nerve function and structural abnormalities (12,13,16), as well as on endoneurial blood flow (15). Clinical studies have shown that ALC is efficacious in the treatment of painful neuropathies (17–19). Based on these data, two multicenter, double-blind, placebo-controlled, randomized, 52-week clinical trials were initiated. The design of the two studies was identical, administering ALC at two doses (500 or 1,000 mg) given three times a day (t.i.d.) for 1 year. Efficacy end points included sural nerve morphometry and sensory and motor nerve conduction velocities, vibration perception threshold, clinical symptom scores, and a visual analogue scale for assessment of the most bothersome symptom at baseline, including neuropathic pain. The data from the two studies were analyzed separately and in combination.

RESEARCH DESIGN AND METHODS

— These were two multicenter, double-blind, placebo-controlled,

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*A complete list of the participating investigators in the Acetyl-L-Carnitine Study Group is available in the APPENDIX.

Abbreviations: ALC, acetyl-L-carnitine; ARI, aldose reductase inhibitor; DPN, diabetic polyneuropathy; NCV, nerve conduction velocity; UCES, U.S.-Canadian-European Study; UCS, U.S.-Canadian Study.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

randomized 52-week prospective studies of type 1 and type 2 diabetic patients with DPN according to the San Antonio criteria (20). Men and nonpregnant women between the ages of 18 and 70 years with diabetes for >1 year and an HbA_{1c} >5.9% were enrolled. Patients with other causes of peripheral neuropathy, significant neurological disorder, alcohol abuse, drug dependency, significant cardiac or hepatic disorders, HIV, or malignant disease were excluded. Women of childbearing age without effective contraception were excluded.

In the two studies, 28 U.S. and Canadian centers (U.S.-Canadian Study [UCS]) and 34 U.S., Canadian, and European centers (U.S.-Canadian-European Study [UCES]) participated (see the APPENDIX), enrolling a total of 1,346 patients entering into the studies.

After obtaining informed consent, eligible patients underwent physical and neurological examinations. Sural nerve conduction velocity (NCV) and vibratory threshold examinations were performed in triplicate (21) during a 4-week run-in period before randomization. The patients had to have a detectable sural nerve amplitude (≥1 μV) to meet the entrance criteria.

Efficacy end point

Morphometric analyses of sural nerves. For logistic reasons, sural nerve biopsies were obtained from U.S. or Canadian patients only from both studies. Of patients who underwent a baseline biopsy, 87% had a second biopsy, yielding 245 evaluable pairs of biopsies. Morphometric parameters included total myelinated fiber number, mean fiber size, fiber density, fiber occupancy, and axon-to-myelin ratio, as previously described (22). These measurements were combined in an O'Brien's average rank score (23). In a separate evaluation, the density of regenerating clusters was assessed ultrastructurally in 209 available biopsy pairs.

Electrophysiological parameters. Measurements were performed in triplicates, at least 1 day apart, at baseline and at completion of the study. The median of the three measurements was used as the value (21). Electrophysiological measurements included bilateral sural NCV and amplitude, peroneal NCV and amplitude on the dominant side, and median motor and sensory NCV and amplitude on the nondominant side. These parameters were combined in an O'Brien average rank score.

Vibration perception. Vibration perception thresholds of the index fingers and great toes were assessed in triplicate (21) at baseline and at the end of the study using a Vibratron (Physitemp Instruments, Clifton, NJ) (24). These measures were combined in an O'Brien's average rank score.

Clinical symptom score and visual analogue scale score. The symptoms reported by the patients at baseline were scored by the investigators on a scale of 0 = no symptoms to 3 = incapacitating symptoms into one of the following categories: pain, numbness, paresthesia, muscle weakness, postural dizziness, problems with sweating, gastrointestinal problems, or sexual dysfunction. In addition, the patients' own assessment of the most troublesome symptom described at baseline was obtained at 26 and 52 weeks. This was indicated on a 10 cm–long visual analogue scale. Pain qualities included throbbing, shooting, and dull pain. Burning sensations were not included.

Population analyzed

All patients who received at least one dose of the study medication and had one valid postrandomization electromyography assessment were included. The methods for analyses were adjusted as follows; for absent electrophysiological data, the "1st percentile procedure" (25) was used. For all other data, the last observation was carried forward. Intention-to-treat patients amounted to 1,257 or 93% of enrolled patients.

The population monitored for safety reasons was 1,335 patients or 99.2% of enrolled patients. Emergent adverse events were classified by body system. Evaluation of the effect of ALC on neuropathic pain was performed on 342 patients (26.7%) who at baseline reported pain as their most bothersome symptom.

Various categories were identified for further analyses. These included the following: age (≤55, >55 years), BMI (≤30, >30 kg/m^2), duration of diabetes (0 to <5 years, 5 to <10 years, ≥10 years), type of diabetes (type 1 or type 2), level of HbA_{1c} (≤8.5%, >8.5%), and adequate drug compliance (<80% vs. ≥80%).

Monitoring the studies

For nerve conduction studies, standardized placements of electrodes and an environmental temperature of 32°C were ascertained. Electrophysiological and vibration perception measurements were standardized between various participating centers. A central reading center was established for all electrophysiological recordings (University of Toronto, Toronto, Canada).

Statistical analysis

Because almost all variables were not normally distributed, rank-transformed data were used in an ANOVA model (for repeated measures when applicable) for all end points. The ANOVA model included factors for treatment, type of disease, and site. The same ANOVA model was used for region-stratified analyses. O'Brien's average rank scores were used to analyze combined end points. All statistical tests were two-sided with a level of significance being <0.05. All values are given as means ± SD.

To account for heterogeneity in the response data for the pain visual analogue scale, a further analysis was performed with an approach using a mixture of linear models (26) to account for such heterogeneities.

RESULTS

Demographic and clinical data

Although the two studies were identical in design, a few demographic parameters differed. Weight and BMI were significantly greater in the UCS ($P < 0.0001$ and $P < 0.0004$, respectively) than in the UCES. On the other hand, the duration of diabetes was significantly longer ($P < 0.0004$) in a smaller proportion of type 2 diabetic ($P < 0.02$) and mainly white ($P < 0.001$) patients in the UCES. These differences became even more apparent when segregated by regions (U.S., Canada, and Europe). Hence, European patients were significantly lighter ($P < 0.0001$) and had a lesser BMI ($P < 0.0004$), longer duration of diabetes ($P < 0.0004$), and higher proportion of type 1 diabetes ($P < 0.001$), whereas U.S. patients were made up of a greater nonwhite population ($P < 0.001$). The differences between U.S. and Canada were small; therefore, the differences between UCS and UCES were mainly due to the European patient cohort in the UCES.

Table 1—*Pain visual analogue scale (UCS, UCES, and pooled cohorts)*

	Placebo	ALC 500 mg t.i.d.	ALC 1,000 mg t.i.d.	ANOVA *P* value (placebo vs. 500 mg ALC)	ANOVA *P* value (placebo vs. 1,000 mg ALC)
UCS					
n	48	61	70		
Baseline	50.40 ± 21.88	57.80 ± 25.92	59.94 ± 24.12	NS	NS
Week 26 change	−10.25 ± 29.45	−17.59 ± 32.53	−23.26 ± 26.33	NS	0.021
Week 52 change	−9.72 ± 31.12	−13.16 ± 32.64	−25.53 ± 28.75	NS	0.024*
UCES					
n	61	43	58		
Baseline	53.21 ± 25.96	48.28 ± 23.30	56.89 ± 26.94	NS	NS
Week 26 change	−18.93 ± 26.20	−14.21 ± 26.82	−21.92 ± 31.28	NS	NS
Week 52 change	−14.51 ± 27.49	−11.84 ± 30.80	−21.75 ± 34.58	NS	NS
Pooled cohorts					
n	109	104	128		
Baseline	51.98 ± 24.18	53.86 ± 25.20	58.56 ± 25.38	NS	NS
Week 26 change	−15.11 ± 27.89	−16.19 ± 30.21	−22.89 ± 28.57	NS	0.031
Week 52 change	−12.40 ± 29.11	−12.61 ± 31.74	−23.82 ± 31.45	NS	0.025†

Data are means ± SD for the change over baseline in intention-to-treat patients. *Repeated-measures ANOVA overall *P* value = 0.031. †Repeated-measures ANOVA overall *P* value = 0.017.

Efficacy end points: nerve biopsy data

Morphometric evaluations of sural nerve biopsies revealed a significant increase in the O'Brien rank score for all biopsy parameters in the 500-mg ALC arm (144.1 ± 28.9 vs. 132.6 ± 37.8, $P = 0.027$), with a significant increase in fiber numbers (−14 ± 197 vs. −98 ± 352; $P = 0.049$) and a significant increase in regenerating clusters (−3.3 ± 8.0 vs. −27.9 ± 9.1; $P = 0.033$). The significant value of the O'Brien rank score was mainly due to the increase in fiber numbers. Patients treated with 1,000 mg ALC t.i.d. were numerically superior to placebo patients, but the differences were not statistically significant.

Electrophysiological data

Individual electrophysiological parameters did not differ significantly between the UCS and UCES, although the O'Brien's rank score for all electrophysiological parameters was significantly lower in the UCES compared with the UCS (112.6 ± 3.45 vs. 124.6 ± 2.53; $P = 0.008$) at baseline. None of the NCV or amplitude measures showed any significant changes in patients taking 500 or 1,000 mg ALC in the combined cohort or in either study group.

Vibration perception threshold

In the UCS cohort, the O'Brien's rank scores for all vibratory parameters revealed significant improvements in patients treated with 1,000 mg ALC t.i.d. when compared with placebo (1,300 ± 571 vs. 1,452 ± 571, $P = 0.007$). Vibration perception improved significantly in the fingers in both the 500- and 1,000-mg ALC t.i.d. groups ($P = 0.040$ and $P = 0.010$) and in the toes in the 1,000-mg t.i.d. group ($P = 0.047$). In the UCES group, patients treated with 1,000 mg ALC t.i.d. showed significant ($P = 0.041$) improvement in vibration perception in the fingers only.

In the region stratified analysis, the improvement in vibration perception of the fingers in European patients were significantly less ($P = 0.041$) than in U.S. and Canadian patients.

Significantly ($P < 0.05$) greater reductions in vibration perception thresholds were seen in the UCS in the following subpopulations: age <55 years, BMI ≤30 kg/m^2, type 2 diabetes, and HbA_{1c} <8.5%. In the UCES, no subpopulation showed significant reductions in vibration perception threshold.

Clinical symptoms score

Evaluation of clinical symptoms in the combined cohorts from the UCS and UCES showed greater mean improvements in both ALC-treated groups compared with placebo at 52 weeks, although no significant differences between either treatment group versus placebo were detected in the O'Brien rank score.

Patient visual analogue scale for pain

Twenty-seven percent of patients reported pain as the most bothersome symptom at baseline (Table 1). The demographics and baseline characteristics of these patients did not differ from those of the entire population (data not shown).

The pooled cohorts treated with 1,000 mg ALC t.i.d. showed significant improvements at both 26 ($P = 0.031$) and 52 ($P = 0.025$) weeks. In the UCS cohort, patients treated with 1,000 mg ALC t.i.d. showed significant improvements at both 26 and 52 weeks ($P = 0.021$ and $P = 0.024$, respectively), whereas in the UCES cohort, no improvements were demonstrated at either time point. The effect sizes for 1,000 mg ALC t.i.d. at 26 and 52 weeks in the combined cohort were 0.28 and 0.38 of the pooled SD, respectively.

In both the UC and UCES cohorts, patients who showed the greatest benefit in pain reduction with 1,000 mg ALC t.i.d. after 52 weeks of treatment were those with type 2 diabetes ($P = 0.055$ and $P = 0.11$, respectively), adequate drug compliance ($P = 0.01$ and 0.37, respectively), and HbA_{1c} >8.5% ($P = 0.009$ and $P = 0.017$, respectively). The mixture of linear models approach yielded the same significant results. In the pooled studies, the responsiveness of pain to ALC treatment was inversely related to duration of diabetes (Fig. 1).

The improvements in pain sensations

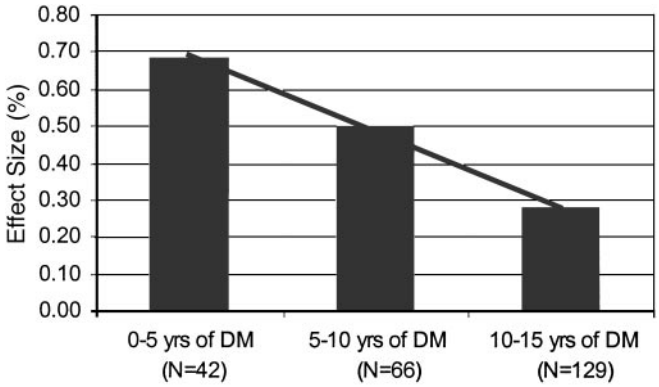

Figure 1—*Effect size of ALC treatment on pain as a function of diabetes (DM) duration. The data represent the pooled cohort.*

were associated with significant improvements in the O'Brien rank score for biopsy parameters in favor of patients treated with 1,000 mg ALC when compared with placebo patients (101.2 ± 31.13 vs. 88.2 ± 31.43, respectively, $P = 0.017$). Specifically, increases in myelinated fiber regeneration ($P = 0.0043$), occupancy ($P = 0.05$), and fiber size ($P = 0.06$) were noted in these patients. No differences versus placebo were noted in these patients with respect to NCV or amplitude.

Finally, patients with pain as the most bothersome symptom showed improvements in the O'Brien rank score for all clinical symptom scores ($P = 0.03$), postural dizziness ($P = 0.03$), and paresthesia ($P = 0.09$).

Adverse events

The most common emergent adverse events were pain, paresthesia, and hyperesthesia. Other events included cardiovascular and gastrointestinal symptoms. There were no safety dropouts. There were nine drug-unrelated deaths. Other dropouts were due to withdrawal of consent and protocol violation. In the total population, pain, paresthesia, and hyperesthesia were reported by significantly fewer patients taking 1,000 mg ALC compared with placebo ($P = 0.026$, $P = 0.023$, and $P = 0.025$, respectively). This was also numerically less in patients taking 500 mg ALC, but the differences did not reach statistical significance. The incidence of other adverse events did not differ between placebo and patients on an active drug.

CONCLUSIONS — In the present study, 1,000 mg ALC t.i.d. for 52 weeks showed beneficial effects on pain in a subgroup (27%) of neuropathic diabetic patients who reported pain as the most bothersome symptom at baseline. Symptomatic relief was present at 26 weeks and was more pronounced in type 2 diabetic patients with suboptimal hyperglycemic control and adequate compliance to treatment. This improvement was associated with improvements in clinical symptom scores and morphometric parameters. Specifically, the latter consisted of increased fiber numbers, clusters of regenerating fibers, and fiber occupancy. However, ALC had no effect on nerve conduction velocities in any of the cohorts.

Neuropathic pain is a common and one of the most troublesome symptoms in DPN. The mechanisms underlying chronic diabetic pain are complex and not fully understood. It can result from overstimulation of nociceptive fibers due to nerve fiber damage (27). Dyck et al. (28) found a correlation between active nerve fiber degeneration and dysesthetic pain. In the present study, ALC treatment presumably inhibited active fiber degeneration, as suggested by the morphometric data, thereby minimizing dysesthetic pain.

Metabolic insults to sensory C- and Aδ-fibers have been invoked in pain (29–31). Damage to axonal membranes of C-fibers causes an increase in Na^+ channels and increased spontaneous firing of C-fibers (27,32). These changes are associated with mitochondrial dysfunction and ischemia-induced exitotoxic effects. After C-fiber degeneration, denervated second-order nociceptive neurons receive collateral branches from Aβ-fibers that release excitatory transmitters. This redistribution of pain processing plays an important role in central sensitization. The present data suggest that ALC has a beneficial effect on small nociceptive fibers. Regeneration and repair of C- and Aδ-fibers are likely to minimize intrinsic excitability (32) and optimize their connectivity with spinal cord interneurons.

These constructs are supported by experimental data showing that ALC improves mitochondrial function, has a beneficial effect on ischemia, and upregulates mGlu2 metabotropic glutamate receptors (14,15,33). Furthermore, ALC upregulates nerve growth factor (34) with beneficial effects on nociceptive substance P expression (35).

The improvement in vibration perception reported here is suggestive of repair of large myelinated fibers. Such effects may also affect the role of Aβ-fibers in central sensitization of pain (29,36,37).

The lack of an effect of ALC on NCV is in retrospect not totally unexpected and is in keeping with previous data from clinical ARI trials. The reason for this may be twofold: *1*) the neuropathy in the present patients was well into the structural phase of DPN with loss of large myelinated fibers evident in the baseline biopsies, and *2*) more importantly, the trial period was too short, not allowing the regenerating clusters to develop into mature myelinated fibers. However, even if this had occurred, it may only have had a small effect on nerve conduction, since regenerated fibers have substantially shorter internodes than the fibers they replace and therefore conduct at a slower velocity, although they may be functional. This anatomical limitation to NCV suggests that NCV may not be the perfect gold standard in these types of clinical trials.

The present findings as well as previous ARI trials (4,6,9) underscore that any intervention in DPN has to be initiated early in the natural history of the disease. Patients who showed the greatest alleviation of pain were those with short duration of diabetes. They were also those who demonstrated improved nerve structure and vibration perception. They were patients in the UCS cohort who had a shorter duration of mainly type 2 diabetes compared with the nonresponders in the UCES group. It is well known that type 2 DPN is less severe and progresses at a slower pace than that of type 1 diabetes (38).

In summary, these analyses have revealed significant improvements in pain and vibratory perceptions associated with

improvements in sural nerve morphometry in patients treated with 1,000 mg ALC t.i.d. for 1 year. These findings were not associated with improvements in NCVs.

In conclusion, the findings suggest that ALC may be of benefit in the treatment of neuropathic pain in patients with DPN. To explore the full effect of ALC on DPN, longer trials initiated at an earlier stage of DPN need to be conducted.

APPENDIX: PARTICIPATING INVESTIGATORS IN THE ACETYL-L-CARNITINE STUDY GROUP

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