

## Effects of L-Acetylcarnitine on Cirrhotic Patients with Hepatic Coma: Randomized Double-Blind, Placebo-Controlled Trial

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**Abstract** Multiple therapeutic modalities have been used to treat hepatic encephalopathy. L-Acetylcarnitine (LAC) is a physiologically active substance that improves both the energetic and the neurotransmission profiles. LAC is able to cross the hematoencephalic barrier and reach the cerebral regions, where the acetylic group may be utilized. The aim of this work was to evaluate the efficacy of LAC in the treatment of hepatic coma in cirrhotic patients. Twenty-four suitably selected patients were enrolled in the study and, following randomization, received either LAC ( $n = 13$ ) or placebo ( $n = 11$ ). Statistically significant differences in neurological findings, as evaluated by the Glasgow Scale, as well as in ammonia serum levels and BUN were found following LAC treatment. In the placebo group we observed two cases of improved neurological findings as well as one case of improved EEG grading. In the other group we observed an improvement of neurological findings and of EEG grade in 10 and 8 subjects, respectively. Noteworthy, seven (54%) patients went from grade 4 down to grade 3, and one from grade 4 down to grade 1. The improvement in the neurological picture was evident at between 1 and 4 hr after the end of

treatment, remaining until 24 hr after. No side effects were observed in our study series. Our study demonstrates that LAC administration improved neurological and biohumoral symptoms in selective cirrhotic patients with hepatic coma.

**Keywords** Hepatic encephalopathy · Hepatic coma · Cirrhosis · L-Acetylcarnitine

### Introduction

Hepatic encephalopathy (HE) is a complex neuropsychiatric disease, which is characterized by disorders of neuromuscular function and mental state, such as sleep disorder, disorientation, somnolence, stupor, and coma [1]. Neurological signs vary with the progression of HE. Hypertonia, hyperreflexia, and a positive Babinsky reflex seem to be present before the occurrence of hyposthenia and lowering of deep tendon reflexes in the later stages of HE. Features of HE may include manifestations of extrapyramidal dysfunction, such as hypomimia, muscular rigidity, bradykinesia, hypokinesia, monotony of speech, and a Parkinson-like tremor [2]. The pathogenesis of HE has been viewed as a multifactorial etiology. Ammonia is a key factor, but cytokines, benzodiazepine-like substances, mercaptans, and products of short-chain fatty acids may also contribute to the neurological disturbance [3–6]. The astrocyte is the key cellular element affected in the brain, and HE can be seen as a disturbance in the relationship of astrocytes with neurons and endothelial cells [7]. Brain edema is observed in HE and recent reports note the presence of cerebral swelling [7, 8]. Astrocyte swelling has been postulated to be a central event in the cascade of neurological disturbances that occur in HE [9, 10].

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L-Acetylcarnitine (LAC) is an ester of the trimethylated amino acid, L-carnitine, and is synthesized in the human brain, liver, and kidney by the enzyme LAC-transferase [11, 12]. The ability to exchange their acetylic active groups gives L-carnitine and LAC a central role in mitochondrial energetic production [13, 14].

LAC is able to cross the hematoencephalic barrier and reach the cerebral regions, where the acetylic group may be utilized [12]. The change-over of acylic group enables this substance to maintain the intramitochondrial salvage pathway, reactivate coenzyme A, reduce intracellular peroxidation and malonyl-aldehyde levels, act as a scavenger, and increase neurotransmitter synthesis due to the structural affinity to acetylcholine. LAC has shown encouraging results in the treatment of degenerative brain disease, when cognitive functions are involved [13, 14]. The aim of this work was to evaluate the effects of LAC in the treatment of cirrhotic patients with hepatic coma, with the primary end point being improvement of neurological function.

## Patients and methods

This protocol was reviewed and accepted by the Medical Ethics Committee of our institution. Informed consent was obtained from the patients' relatives.

### Inclusion criteria

Patients enrolled were between 18 and 70 years of age, with biopsy-verified liver cirrhosis at stage 4 HE [15, 16].

### Exclusion Criteria

Exclusion criteria were as follows: (a) intake of synthetic benzodiazepines or other sedative drugs in the previous 7 days; (b) alcohol abuse in the previous 10 days; (c) uncontrolled diabetes mellitus; (d) final-stage severe kidney failure, as defined by a BUN >90 mg/dl and/or serum creatinine >4 mg/dl; (e) severe respiratory failure, as defined by  $PO_2$  <60 mm Hg and/or  $PCO_2$  <50 mm Hg; (f) acidosis, with blood pH <7.30; (g) pre-existing neurological disease; (h) heart failure; (i) hemodynamic instability; (j) intake of any drug for the specific treatment of HE in the previous 24 hr (except lactulose); (k) refusal to sign informed consent by patients' relative; and (l) endocrine diseases.

### Study design

After a 12-hr stabilization period and after a stratification process for Child- Pugh score and for clinical grading of coma, a computer-generated randomization schedule was used to assign the patients to the LAC or placebo group.

A neurological assessment was performed 30 min before and 30 min after drug administration. All evaluations were made blindly and independently by two observers (L.R., I.V.). Two physicians evaluated all the patients; each physician evaluated the same patient before and after treatment. Patients in group A received 4 g LAC in 500 ml of a 5% glycosylated solution (g.s.), while group B received only the 5% g.s., intravenously over 3 hr, once a day for 3 days. Two sets of vials (aqueous solution or active drug) were prepared for each patient for administration according to the randomization design. Furthermore, all patients received lactulose (30 ml thrice a day, per os) for 30 days. No other drug was administered throughout the study.

The neurological assessment was conducted taking into consideration the following parameters: verbal ability, eye opening, pupillary light reflex, corneal reflex, spontaneous eye movements, oculocephalic reflex, motor response, and pattern of respiration [16]. Improvement in clinical neurological function was defined as an improvement in two of the eight items within 2 hr of drug administration.

Clinical grading of coma was as follows: grade 1 was defined as the presence of euphoria or depression, mild confusion, slowness, and disorders in sleep rhythm; grade 2, as the presence of drowsiness, inappropriate behavior, and enhancement of state 1 signs and symptoms; grade 3, as the presence of stupor, sleeping almost all day, incoherent speech, and marked confusion; and grade 4, as the presence of coma with (a) coordinated response to painful stimulus, (b) hyperextension and pronosupination after a painful stimulus, or (c) no response to a painful stimulus [17]. Neurological assessment was repeated every 15 min up to 6 hr after placebo and LAC administration.

We used the Glasgow [25] coma scale (score range, 3–13) to evaluate the following parameters.

- (a) Eye response: 1 = no response; 2 = open in response to pain; 3 = open under verbal command; 4 = open without a stimulus.
- (b) Motor response: 1 = no response; 2 = extension in response to pain; 3 = flexion in response to pain; 4 = appropriate motor response to pain; 5 = execution of commands.
- (c) Verbal response: 1 = no response; 2 = grunting in response to pain; 3 = noncoherent speech; 4 = understandable speech.

### EEG

EEG tracking was performed 15 min before and 15 min after drug administration. Two observers observed modifications in EEG tracking blindly and independently (L.R., RR). EEG grading of HE was as follows.

Grade 0: HE was defined as the presence of background activity (alpha rhythm).

Grade 1: An alpha rhythm with some scattered theta waves.

Grade 2: Background activity of theta rhythm mixed with some delta and alpha waves.

Grade 3: Background of polymorphic delta activity characterized by a high amplitude with spontaneous variability.

Grade 4: Delta activity characterized by a low amplitude.

#### Venous ammonia concentration

Serum levels of ammonia were evaluated by an enzymatic method employing glutamate dehydrogenase in a rapid and interference-free photometric determination (340 nm) of  $\text{NH}_4^+$  in blood plasma according to the De Fonseca–Wollheim method [18]. Within 15 min from withdrawal, the blood sample was frozen ( $-20^\circ\text{C}$ ) for later determination of  $\text{NH}_4^+$ .

#### Safety parameters

Safety parameters evaluated included blood tests (serum hemoglobin, hematocrit, complete blood cell count) and liver function tests (serum alanine amino transferase, aspartate amino transferase,  $\gamma$ -glutamyl transpeptidase, cholinesterase activity, serum bilirubin concentrations, prothrombin time, and partial thromboplastin time) carried out within 6 hr before and after treatment each day.

#### Statistical analysis

Data were analyzed according to the “intention to treat” principle. Patients’ characteristics at randomization before treatment were compared by means of Student’s test. Descriptive statistics were prepared from the study sample and the results are expressed as mean  $\pm$  standard deviation.

Statistical significance in contingency tables was evaluated using the chi-square and Fisher exact tests. Student’s test for unpaired data, one-way ANOVA, and Mann-Whitney rank sum test were used for comparisons of continuous variables. Statistical analysis was performed using tests for repeated measures as well as by controls for multiple comparisons with correction by Duncan procedure.

The hypothesis was that LAC improves neurological symptoms in 80% of patients, compared with a 10% improvement in the placebo group. The  $\alpha$  error was 0.05 and the  $\beta$  error was 0.2 (two-sided test). We calculated that we would have to evaluate nine patients in each group to test this hypothesis. We further calculated that, because of an estimated 10% dropout rate after randomization, at least 10 patients would have to be included in each group.

## Results

Between January 1997 and July 2002, 65 cirrhotic patients in hepatic coma were evaluated at our institution; 41 patients were excluded from the trial because of the presence of at least one of the exclusion criteria (15 due to refusal by relative to sign informed consent form, 6 due to respiratory failure, 6 due to renal failure, 5 due to heart failure, 3 due to endocrine disease, and 6 due to death). Twenty-four patients were enrolled in the study and, after randomization, received LAC ( $n = 13$ ) or placebo ( $n = 11$ ) (Table 1). Clinical characteristics of patients at randomization are presented for both groups in Table 1. The two groups were similar with regard to age, sex, pathogenesis of cirrhosis, and severity of liver disease.

The characteristics of hepatic coma at randomization are summarized in Table 2. Baseline neurological scores, duration of coma before randomization, and precipitating factors were similar in both groups. In the LAC group the

**Table 1** Patients’ baseline characteristics (biochemical parameters 6 hr before drug administration)

Parameter	L-Acetylcarnitine	Placebo	<i>P</i> value
Age	51.4 $\pm$ 9.1	50.2 $\pm$ 8.9	NS
Males/females	9/4	7/4	NS
Alcoholic cirrhosis	3	3	NS
Posthepatitis cirrhosis	6	5	NS
Unknown cirrhosis	4	3	NS
Child-Pugh score	11.1 $\pm$ 1.4	11.4 $\pm$ 1.1	NS
Serum albumin	2.4 $\pm$ 0.61	2.38 $\pm$ 0.64	NS
Serum bilirubin	3.81 $\pm$ 2.62	3.79 $\pm$ 2.84	NS
Serum BUN	74.2 $\pm$ 8.6	74.1 $\pm$ 8.8	NS
Serum creatinine	1.44 $\pm$ 0.55	1.36 $\pm$ 0.59	NS
Serum ammonia	125.4 $\pm$ 30.6	128 $\pm$ 28.6	NS
Serum $\text{PO}_2$	99.4 $\pm$ 23.8	88.2 $\pm$ 20.9	NS
Serum $\text{PCO}_2$	27.6 $\pm$ 3.9	27.9 $\pm$ 3.5	NS
Blood pH	7.44 $\pm$ 0.05	7.42 $\pm$ 0.03	NS

**Table 2** Baseline coma characteristics at randomization

Parameter	L-Acetylcarnitine group	Placebo group	<i>P</i> value
Initial neurological score	10.8 ± 1.4	10.6 ± 1.7	NS
Hemorrhage	5	4	NS
Sepsis	1	1	NS
Dehydration	1	1	NS
Surgery	1	1	NS
Unknown	5	4	NS
Duration of coma before randomization, hr (Range)	2.4 ± 1.8(1.10–5.40)	2.00 ± 1.9 (1.20–5.10)	

hemorrhage in five patients was due to variceal bleed, the sepsis in one patient was due to urinary tract infection, and in one patient there was surgical intervention for inguinal hernia. In the placebo group four patients also had hemorrhage due to variceal bleed, one patient had sepsis due to pulmonary infection, and one patient had surgery for umbilical hernia.

In the group treated with placebo we observed two cases of improvement of neurological findings as well as one case of improvement of EEG grading (Table 3). In the LAC group we observed an improvement of neurological findings and of EEG grade in 10 and 8 subjects, respectively. Noteworthy, seven (54%) patients went from grade 4 down to grade 3, and 1 patient from grade 4 to grade 1 (Table 3). Furthermore, Glasgow score ( $P < 0.0001$ ; 95% CI = 2.72–6.68), serum ammonia ( $P < 0.01$ ; 95% CI = 7.04–55.36), and BUN ( $P < 0.01$ ; 95% CI = 1.85–16.75) changed significantly. The improvement in the neurological picture was evident at from 1 to 4 hr after the end of the treatment, remaining until 24 hr after. Comparison between the two groups at the end of treatment showed a decrease in Glasgow score ( $P < 0.006$ ; 95% CI = -5.53 to -1.07), serum ammonia ( $P < 0.009$ ; 95% CI = -44.18 to -3.58), and serum BUN ( $P < 0.000$ ; 95% CI = 18.86 to 32.94) (Table 3).

No side effects were observed in our study series.

## Discussion

Multiple therapeutic modalities have been used to treat HE. Meta-analyses are difficult to perform due to wide heterogeneity among studies, reflecting problems in the choice of

placebo and the control of precipitating factors, as well as the difficulty of grading degrees of encephalopathy [19]. Therapeutic approaches have been based mostly on the correction of trigger factors, on drugs that reduce the ammonia production within the bowel [20], on branched chain amino acid utilization and finally on benzodiazepine antagonists [21–25].

In rodents used as model organisms, carnitine supplementation appeared to prevent ammonia toxicity on three levels: (i) activation of urea cycle enzymes, (ii) interaction with glutamate receptors, and (iii) reduction of free radicals [26]. Carnitine therefore may suppress the accumulation of acetyl-CoA and short-chain acyl CoA esters, which are mainly degradation products of branched-chain amino acids. The acetyl-CoA/CoASH ratio is an important regulating factor of the oxidation of pyruvate,  $\alpha$ -ketoglutarate, and fatty acids. Therefore carnitine may be effective at limiting the demands placed on cirrhotic subjects by acute stressors, such as a sudden increase in physical activity, an immunologic challenge, or acute malnutrition [27].

To date, few studies have been performed on L-carnitine and LAC as therapeutic tools in HE [28–31]. Our study has demonstrated that the administration of LAC may improve neurological and biohumoral symptoms in selected cirrhotic patients with hepatic coma. Since no adverse events were observed, we suggest that the LAC treatment is both effective and safe. This substance acts either on energy producing mechanisms of mitochondria (correcting the previous energy deficiency) or by reduction of serum  $\text{NH}_4^+$  levels. The latter was observed significantly in our patients, especially comparing the values obtained with LAC versus placebo.

**Table 3** Main parameters at the start and the end of treatment in the two groups

Parameter	Before LAC treatment	After LAC treatment	Before placebo treatment	After placebo treatment
Glasgow score	10.6 ± 1.3 <sup>a</sup>	5.9 ± 3.2 <sup>a,c</sup>	10.4 ± 1.4 <sup>b</sup>	9.2 ± 1.7 <sup>b,c</sup>
EEG grade 4	13/13	5/13 (38%)	11/11	10/11 (91%)
EEG grade 3	–	7/13 (54%)	–	1/11
EEG grade 2	–	1/13 (8%)	–	–
Ammonia	109 ± 31.4 <sup>d</sup>	77.8 ± 28.2 <sup>d,f</sup>	106.1 ± 30.2 <sup>e</sup>	98.1 ± 28 <sup>e,f</sup>
BUN	75.4 ± 10.1 <sup>g</sup>	66.1 ± 8.2 <sup>g,i</sup>	73.8 ± 9.7 <sup>h</sup>	70.2 ± 8.4 <sup>h,i</sup>

Note. LAC, L-acetylcarnitine.

<sup>a</sup> $P = 0.000$ ; <sup>b</sup> $P = 0.086$ ; <sup>c</sup> $P = 0.006$ ; <sup>d</sup> $P = 0.014$ ; <sup>e</sup> $P = 0.527$ ; <sup>f</sup> $P = 0.092$ ; <sup>g</sup> $P = 0.017$ ; <sup>h</sup> $P = 0.363$ ; <sup>i</sup> $P = 0.240$ .

These values were confirmed by variations in BUN. Brain toxicity of  $\text{NH}_4^+$  may be explained by energy deficiency. In fact,  $\text{NH}_4^+$  is metabolized by brain cells with glutamate consumption, resulting in glutamine production [32]; this reaction may induce a reduction in aspartate and glutamate, a depletion of acetyl-CoA, a slowing of Krebs cycle flux, and a deficiency of cell energy production. LAC may induce an increase in acetyl-CoA and, consequently, an amelioration of Krebs cycle flux.

Apart from modalities of LAC action at either the biohumoral or the intracellular level, it is important to highlight that we observed an improvement in Glasgow scale mean values in the patients treated with LAC versus those treated with placebo, confirmed by EEG findings.

A study carried out on autopsied brain tissue from humans and mice with acute liver failure, resulting from hepatic devascularization at precoma and coma stages of encephalopathy, revealed cytotoxic edema consisting of cytoplasm in perineuronal and perivascular processes of astrocytes [33]. Studies conducted on patients with mental degenerative and vascular disorders related to aging showed an improvement after a course of LAC administration [13, 14].

The benefit of LAC not only lies in its energy production and  $\text{NH}_4^+$  metabolism, but also may be due to its activity on short-chain fatty acids, called precipitating factors of hepatic coma. LAC allows the uptake of fatty acids into the mitochondria, reducing their toxicity. Finally, considering that LAC exerts its activity at the mitochondrial level, it is possible that it interferes with peripheral-type benzodiazepine receptors, which are located in the mitochondria.

In conclusion, this study has shown a clinically significant effect of LAC on objective signs of hepatic coma in a highly selected study series.

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