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L-Carnitine in the Treatment of Mild or Moderate Hepatic Encephalopathy

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Key Words
Hepatic encephalopathy · *L*-Carnitine treatment

Abstract
Hepatic encephalopathy (HE) is one of the major complications of cirrhosis. Experimental and clinical findings observed in liver, muscle and brain have provided new insights into the ammonia mechanism of action. *L*-Carnitine (LC), inducing ureagenesis, may decrease blood and brain ammonia levels. 120 patients meeting inclusion criteria were randomized either to a treatment for 60 days with LC or placebo (2 g twice a day). Previous studies have reported a significant protective effect of LC in mice and rats, which is associated with a significant reduction of blood and brain ammonia concentration, suggesting an action of LC either at peripheral or central sites. Results of our study show a protective effect of LC in ammonia-precipitated encephalopathy in cirrhotic patients. Either in subjects with HE 1 or 2 we observed a significant reduction at day 30 and more markedly at day 60 of treatment. A significant therapeutic effect of LC was also observed in the NCT-A, which is an accepted and reliable psychometric test for the assessment of mental function in cirrhotic patients with HE.

Introduction

Hepatic encephalopathy (HE) is one of the major complications of cirrhosis. Clinical grading is based on impaired mental function, neurological disorders and altered states of consciousness, to stupor and coma. Pathogenesis of this common syndrome remains partially unknown. Hyperammoniemia, due to the several mechanisms proposed, is one of the main pathogenetic factors in the development of HE, as shown by the association of increased serum ammonia levels with impairment of mental function in patients with liver failure. Experimental and clinical findings observed in liver, muscle and brain have provided new insights into the ammonia mechanism of action. In fact, these organs play a fundamental role in the removal of ammonia in cases of hyperammoniemia [1, 2].

A decrease in the muscle mass is a jeopardizing factor for the development of encephalopathy [3]. Muscle mass loss is also associated with a decreased ability to change ammonia in glutamine. All morphological and functional characteristics of the brain in experimental models and humans rule out an initial involvement of neurons in HE, the astrocytes being the first target of the disease [4]. As showed by Ullian et al. [5], these cells play a key role in the correct development and function of neurons. In HE, no

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morphological neuronal alterations are detected, changes in astrocyte characteristics being present (Alzheimer type II cells) [6]. The majority of therapeutic measures currently in use are therefore aimed at reduction of serum ammonia levels, by decreasing enteric ammonia production [7].

Carnitine is a natural substance involved in regulating substrate flux and energy balance across cell membranes. It is a cofactor for the shuttle mechanism whereby long-chain fatty acids are transformed into *L*-acetylcarnitine metabolites and may be carried out into mitochondria in order to be part of β-oxidation [8]. Furthermore, carnitine and the carnitine acetyltransferase are involved in the following reaction: acetyl-coenzyme A + carnitine = acetylcarnitine + coenzyme A. This reaction modulates the intracellular concentration of coenzyme A and acetyl-CoA. By this reaction, carnitine produces free CoA for other metabolic reactions and reduces the ratio of acetyl-CoA to CoA. This reduction stimulates the activity of pyruvate dehydrogenase and, thus, enhances the oxidative use of glucose.

O'Connor et al. [9] showed a protective effect of *L*-carnitine (LC) administration to mice 1 h before a lethal injection of ammonium acetate. Therrien et al. [10] reported that LC in the portocaval shunted rat prevented not only the development of severe encephalopathy, but also reduced mortality. It was suggested that LC, inducing ureagenesis, may decrease blood and brain ammonia levels. In order to assess the clinical efficacy of LC in the treatment of HE, a randomized, double-blind, placebo-controlled study with oral administration in cirrhotic patients with hyperammoniemia and chronic symptoms has been carried out.

The aim of our study was to evaluate in a practice-adapted design the influence of LC using the number connection test A (NCT-A), mental conditions and ammonia effects.

Patients and Methods

120 randomly selected patients (9 alcoholics, 33 hepatitis B virus infected, 63 hepatitis C virus infected, 15 cryptogenetic cirrhosis) met the following inclusion criteria and were enrolled in the study: (1) chronic hepatitis with spontaneous manifest HE (mental state grade 1 or 2 according to the West Haven criteria) and an NCT-A performance time >30 s; (2) hyperammoniemia (venous ammonia concentration >50 mmol/l), and (3) cooperative, hospitalized adult patients with liver cirrhosis diagnosed by clinical, histological and ultrasonographic findings.

Exclusion criteria were the following: (1) major complications of portal hypertension, such as gastrointestinal blood loss, hepatorenal syndrome or bacterial peritonitis; (2) acute superimposed liver injury; (3) patient with other neurological disease and metabolic disorders such as alcoholism, diabetes mellitus, unbalanced heart failure and/or respiratory failure or end-stage renal disease; (4) severe HE (mental state grade 3–4); (5) administration of anti-HE medications such as neomycin, lactulose, lactitol, branched-chain amino acids; (6) any additional precipitating factors such as high protein intake (additional high-protein meals), constipation or intake of psychostimulants, sedatives, antidepressants, benzodiazepines, or benzodiazepine antagonists (flumazenil), and (7) patients with fever, sepsis or shock were also excluded to avoid variations caused by body temperature.

Study Design

Patients meeting inclusion criteria were randomized in a double-blind fashion to either a treatment for 60 days with LC (2 g twice a day) (group A) or placebo (which were identical in appearance and packaging) (group B). Randomization was based on a computer-generated list. The consumption of oral LC at a dose of 4 g/day divided into two doses (2 g in the morning and 2 g in the evening) for 60 days met the 100% of compliance. A minimum of 210 pills (each pill 1 g) was considered as good compliance. Concomitant medications (whose administration continued throughout the study) included diuretics (36 of group A and 30 of group B) and β-blockers (27 of group A and 25 of group B).

All study series were subdivided into groups belonging to HE 1 or HE 2 according to the initial HE grade (West Haven criteria). Group A was composed of patients with initial HE 1 (LC 35 patients, placebo 33 patients); group B with initial HE 2 (LC 25 patients, placebo 27 patients). The effectiveness of therapy was compared and evaluated separately in the different subgroups.

The groups were homogeneous with regard to anamnestic and diagnostic criteria. Differences in the composition of the two groups with respect to precipitant factors may be minimized, because the patient population was well defined by inclusion and exclusion criteria. Any pathological, clinical or laboratory findings observed during the study were monitored and documented until their normalization.

NCT-A

The NCT-A measures cognitive and motor ability, and consists of a combination of number series on a sheet with best celerity possible. A decrease in the time employed to complete the NCT-A reflects an improvement of the neurological function.

Venous Ammonia Concentration

The blood ammonia levels were evaluated by the enzymatic determination using glutamate dehydrogenase in a rapid and interference-free photometric determination (340 nm) of NH_4^+ in native blood plasma according to the De Fonseca-Wollheim method [12]. The blood sample was immediately taken after withdrawal to the laboratory for immediate (within 15 min) determination of $NH4_4^+$.

Hepatic Encephalopathy

Encephalopathy grade was diagnosed on the basis of the evaluation of consciousness, intellectual functions, behavior and neuromuscular functions according to the West Haven criteria introduced by Conn and Liebenthal [13, 14].

Table 1. Baseline characteristics of patients

Parameter	Carnitine group (A)	Placebo group (B)
Male/female	40/20	38/22
Age	51.7 ± 11.8	52.4 ± 10.4
Cirrhosis etiology		
Alcohol	4	5
Post-hepatitis B	16	17
Post-hepatitis C	32	31
Cryptogenetic	8	7
Child-Pugh class		
A	29	30
B	32	31
C	9	9
Prothrombin time, %	60.4 ± 6.7	61.2 ± 6.9
Serum albumin level, g/dl	2.7 ± 0.6	2.8 ± 0.5
Serum bilirubin level, mg/dl	3.8 ± 1.4	3.6 ± 1.5
Serum alanine aminotransferase level, IU/l	128 ± 61	120 ± 63
Blood urea nitrogen, mg/dl	44 ± 10	43 ± 12
Serum creatinine level, mg/dl	1 ± 0.15	0.98 ± 0.14
Natriemia, mEq/l	133 ± 3.4	132 ± 4.7

Safety Parameters

Safety parameters included blood tests (hemoglobin, hematocrit, white and red blood cell count, platelet count) and liver function tests (alanine aminotransferase, aspartate aminotransferase, γ-glutamyltranspeptidase, cholinesterase activity, serum bilirubin, serum albumin concentrations, prothrombin time and partial thromboplastin time) on days 0, 30 and 60.

Statistical Analysis

Descriptive statistics were prepared from the study sample and results were expressed as means ± SD. Statistical significance in contingency tables was evaluated using χ^2 test and Fisher's exact test. Student's test for unpaired data, one-way ANOVA and Mann-Whitney rank sum test were used for comparisons of continuous variables. Statistical analyses were performed using appropriate tests for repeated measures as well as by controlling for multiple comparisons with correction of the Duncan procedure. The study protocol was received and approved by the Institutional Review Board of the Hospital following the guidelines of the 1975 Declaration of Helsinki.

Results

Baseline Values

The two groups were homogeneous for demographic characteristic, etiology, casting of disease and Child-Pugh grade. Serum NH_4^+ fasting concentrations were not significantly different before the treatment. NCT-A did not show significant differences at baseline.

L-*Carnitine Treatment*

The patients treated with carnitine either in group HE 1 or in group HE 2 showed fasting statistical significant differences in serum NH_4^+ levels. In group HE 1, after 30 days of treatment, p was 0.003 (CI 6.37–30.43), being 0.000 (CI 27.82–48.98) after 60 days compared to baseline values and 0.000 (CI 11.14–28.86) compared to day 30. In group HE 2, p was 0.000 (CI 15.76–40.04) after 30 days, being 0.000 (CI 32.96–53.24) after 60 days compared to baseline values and 0.001 (CI 6.01–24.39) compared to day 30 of treatment respectively. With reference to NCT-A, in group HE 1 we observed a p of 0.000 (CI 6.23–19.17) after 30 days and 0.000 (CI 17.78–30.62) after 60 days compared to baseline values as well as 0.000 (CI 5.15–17.85) with respect to day 30 of treatment respectively. In group HE 2 we found a p of 0.000 (CI 7.91–21.89) after 30 days and 0.000 (CI 20.20–34.20) after 60 days compared to baseline values and 0.000 (CI 5.63–18.97) with respect to day 30 of treatment.

Placebo Treatment

No statistical difference was found in these patients at days 30 and 60 with respect to baseline and day 30 values respectively in both groups. In fact, for fasting serum NH_4^+ levels, in group HE 1 we found a p of 0.477 after 30 days (CI –7.63 to 16.23), after 60 days a p of 0.419 (CI –7.21 to 17.21) with respect to baseline values and 0.905 compared to values of day 30 of treatment (CI –10.87 to 12.27).

In group HE 2 we found a p of 0.396 (CI –7.01 to 17.61) after 30 days and of 0.303 (CI –5.95 to 18.95) after 60 days with respect to baseline and 0.832 (CI –10.01 to 12.41) with respect to values of day 30 respectively. For

Table 2. Comparison between evaluated parameters of the two groups (days of treatment)

		Carnitine group			Placebo group		
		0 days	30 days	60 days	0 days	30 days	60 days
NH_4^+ fasting	HE 1	80.2 ± 36.9	61.8 ± 29.2	41.8 ± 18.7	80.7 ± 34.7	76.4 ± 31.2	75.7 ± 32.8
	HE 2	88.7 ± 35.6	60.8 ± 31.4	45.6 ± 17.5	86.7 ± 37.2	81.4 ± 30.6	80.2 ± 31.4
NCT-A	HE 1	62.8 ± 18.1	50.1 ± 17.7	38.6 ± 17.4	60.7 ± 20.3	58.7 ± 19.4	56.7 ± 20.7
	HE 2	66.4 ± 20.2	51.5 ± 18.4	39.2 ± 18.5	65.8 ± 21.4	65.1 ± 19.7	63.2 ± 23.1

NCT-A, in group HE 1 we found a p of 0.582 after 30 days (CI –5.18 to 9.18) and of 0.287 (CI –3.41 to 11.41) after 60 days compared to baseline values and 0.586 (CI –5.25 to 9.25) with respect to values of day 30. Following the same sequence of the previously listed parameters, in group HE 2 we found a p of 0.852 (CI –6.74 to 8.14) after 30 days, 0.524 (CI –5.45 to 10.65) after 60 days with respect to basal conditions and 0.629 (CI –5.86 to 9.66) compared to day 30 of administration.

Comparison between Treatments

In group HE 1 for NH_4^+ at day 30 of administration, the patients treated with carnitine showed a response statistically significant with respect to placebo (p = 0.009 CI –25.252 to –3.68); in group HE 2, at day 30 of administration p was 0.000 (CI –31.81 to –9.39). For NCT-A, in group HE 1 at day 30 of administration a statistical difference was found with p 0.012 (CI –15.31 to –1.89), while in group HE 2 at day 30 of administration p was 0.000 (CI –20.49 to –6.71) in favor of carnitine patients. At day 60 for NH_4^+ in group HE 1, carnitine-treated patients showed a better significant response than placebo patients, p was 0.000 (CI –43.55 to –24.25); in group HE 2, p was 0.000 (CI –43.73 to –25.41) in favor of carnitine. About NCT-A, at day 60 the response was strikingly in favor of carnitine-treated patients: in group HE 1, p was 0.000 (CI –25.01 to –11.19), while in group HE 2, p was 0.000 (CI –31.57 to –16.43).

Adverse Events

Both LC and placebo were well tolerated in 100% of patients. In the group treated with LC, 1 patient complained of nausea, 2 of slight headache and 2 of abdominal pain. In placebo group, 2 patients complained of diarrhea and 1 of moderate headache. Nobody withdrew from the planned treatment.

Discussion

Several pathogenetic mechanisms have been suggested to explain the onset and progression of HE. The most ancient hypothesis is linked to the neurotoxicity of ammonia. The majority of blood NH_4^+ amount results from muscle protein catabolism at intestinal level; the remnant is produced by the action of colic bacteria on the natrium present in digested foods. Ammonia is vehicled to the liver throughout the portal flux and is normally eliminated as urea. The liver damage or the presence of portosystemic shunts increase its serum levels [15, 16]. The exceeding ammonia is eliminated from the blood by transforming of glutamate into glutamine in skeletal muscle as well as central nervous system. Neurotoxicity of ammonia is probably due to a direct action on neurons, because the reduction of glutamate and the increase of glutamine may induce a swelling of the astrocytes [16].

Previous studies have reported a significant protective effect of LC in mice and rats, which is associated with a significant reduction of blood and brain ammonia concentration [9, 17], suggesting an action of LC either at peripheral or central sites. In the study by O'Connor et al. [9], blood urea concentrations were significantly increased and inversely related to the lowering of blood ammonia after one carnitine administration to normal mice to whom toxic doses of ammonia were administered. This fact suggests that LC's effect in these animals was caused, at least partially, by an action on hepatic urea synthesis. Results of our study show a protective effect of LC in ammonia-precipitated encephalopathy in cirrhotic patients. Either in subjects with HE 1 or HE 2, we observed a significant reduction at day 30 and more markedly at day 60 of treatment. A significant therapeutic effect of carnitine was also observed in the NCT-A, which is an accepted and reliable psychometric test for the assessment of mental function in cirrhotic patients with HE [13, 18]. LC crosses the hematoencephalic barrier

slowly (brain uptake index 5.5%) but in spite of this fact, its amount in the brain is relatively large [19]. In the study of Therrien et al. [20], the protective effect of LC was accompanied by a significant attenuation of the increased cerebrospinal fluid and brain alanine as well as cerebrospinal fluid lactate content, caused by ammonium acetate administration. This fact suggests that mitochondria respiration is at least partially restored in LC-treated animals. The possible beneficial effect of carnitine may be related to an improved pyruvate oxidation, Krebs cycle and flux through glutamate dehydrogenase. The latter could then explain the lowering of blood ammonia levels that follows LC administration. In conclusion, this study demonstrates a clinically significant effect of LC on mental conditions and ammonia levels in patients with mild or moderate HE.

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