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ORIGINAL PAPER

Acetyl-L-carnitine improves cognitive functions in severe hepatic encephalopathy: a randomized and controlled clinical trial

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Abstract The aim of this study was to investigate the effects of ALC treatment on cognitive functions in patients with severe hepatic encephalopathy. This was a randomized, double-blind, placebo-controlled study. 61 patients with severe hepatic encephalopathy were recruited to the study. The 2 groups received either 2 g ALC twice a day (n=30) or placebo (n=30) for 90 days. Clinical and laboratory assessment, psychometric tests and automated electroencephalogram (EEG) analysis were performed for all patients. At the end of the study period, between the 2 groups we observed a significant difference in Everyday Memory Questionnaire −23.9 vs 4.4 (p<0.001), Logical Memory (Paragraph recall) test 22.3 vs 0.7 (p<0.001), Trail Making Test A −7.5 vs −2.6 (p<0.001), Trail Making Test B −10.5 vs −3.1 (p<0.001), Controlled Oral Word Association Test 4.2 vs 0.5 (p<0.001), Hooper test 2.6 vs 0.1 (p<0.05), Judgement of line orientation 2.8 vs 0.3 (p<0.001), Digit Cancellation time −24.5 vs −2.4 (p<0.001), NH_4^+ 30.5 vs 13.5 (p<0.001), prothrombin time 2 vs 2.4 (p<0.05), alanine transaminase −10.7 vs −13.6 (p<0.001). 88% of patients treated with ALC vs 72% of patients treated with placebo showed a significant improvement in EEG. The improvement of cognitive deficits, the reduction of ammonia, and the modification of EEG in patients treated with ALC suggest that ALC could represent a new tool in the treatment of severe hepatic encephalopathy.

Keywords Acetyl-L-carnitine · L-carnitine · Severe hepatic encephalopathy · Cognitive functions

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Introduction

Hepatic encephalopathy (HE) is a reversible state of impaired cognitive function or altered consciousness which occurs in subjects with liver disease or portal systemic shunts (Voigt and Conn 1995). The severe HE may progress within a matter of hours from a mild confusional state to deep coma. Severe HE (grade 3 of the West Haven grading scale) is characterized by severe disorders of consciousness, intellectual function, personal and behaviour and neuromuscular abnormalities (Table 1). Signs of raised intracranial pressure (bradycardia, hypertension, dilated pupils) are common in patients with severe encephalopathy. Asterixis (liver flap) should be sought and tendon reflexes tested; the latter are often increased, unlike in many patients who are drowsy. The toxins possibly implicated in aetiology of HE are ammonia, false neurotransmitter (octopamine, phenylethanolamine) gamma-amino butyric acid, short chain fatty acids, mercaptanes neurosteroids and manganese (Butterworth 2001). Exhalation of unmetabolized mercaptans leads to fetor hepaticus (a sweet musty smell on the breath). It therefore appears that, although ammonia probably has a

Table 1 Disorders in patients with Severe Hepatic Encephalopathy (grade 3 of the West Haven grading scale)

Consciousness	somnolence
	confusion
	semistupor
Intellectual function	disorientation in space
	amnesia for recent and past events
	inability to perform calculations
Personality and behaviour	strange behaviour
	paranoia or anger
	rage
Neuromuscular abnormalities	asterixis
	hyperactive reflexes
	nistagmus
	Babinski myoclonus

central role in the pathogenesis of HE, its effects are mediated through alteration of a number of neurotransmitter concentration and cellular changes of the astrocytes along with an alteration of the blood–brain barrier. Recent research has confirmed that ammonia affects a number of neurotransmitter systems and exerts its effect through its products of metabolism (e.g. glutamate and glutamine). Increased glutamine in astrocytes causes osmotic stress, leading to cellular swelling and cellular change, termed Alzheimer type 2 astrocytosis. In addition, GABA-ergic tone and peripheral benzodiazepine receptor binding increased in HE with serotonin and dopamine neurotransmission also previously shown to be abnormal. In recent years L-carnitine has become more prevalent in therapies aimed at improving mitochondrial energy metabolism and it is beneficial in elderly subjects and in HE patients (Malaguarnera et al. 2003, 2005, 2007, 2008). Acylcarnitine have shown beneficial effects in the treatment of aging, chronic degenerative diseases and slowing the progression of mental deterioration in AD (Spagnoli et al. 1991). L-carnitines are ubiquitously occurring trimethylated aminoacids that play an important role in the transport of long-chain fatty acids across the inner mitochondrial membrane (Bremer 1983) and are essential for energy production through fatty acid metabolism. Acetyl-L-carnitine (ALC) represents an acetylated from L-carnitine and is the most important carnitine ester found in the tissues of animals. ALC has a positive role in maintaining the functional activity of various organs in various pathologies and in the course of aging. ALC is synthesized in mitochondria by a reversible acetylation process of L-carnitine catabolised by the acetyl-transferase. ALC is able to cross the blood brain barrier and reaches the nervous areas where the linked acetylic group may be delivered. Some of ALC's proposed neuroprotective benefits involve improved mitochondrial energetic and function, antioxidant activity, stabilization of membranes, protein and gene expression modulation and enhancement of cholinergic neurotransmission. To assess the clinical efficacy of ALC in the treatment of severe HE (grade 3 of the West Haven grading scale), we performed a randomized, double blind placebo-controlled study administering ALC to cirrhotic patients, evaluating the effects on ammonia levels and performance in cognitive functions.

Materials and methods

Between July 2002 and December 2006, a total of 68 consecutive outpatients with severe HE (grade 3 of the West Haven grading scale) with hepatic cirrhosis, were screened.

The West Haven grading scale will be used to describe the stages of HE unless otherwise stated. Of the 68 patients approached, 3 were not eligible, 3 refused participation, 1 died. The remaining 61 patients agreed to participate to the study. Informed consent was obtained from patients and patients' relatives as approved by the Institutional Review Board at Cannizzaro Hospital in Catania following the guidelines of the 1975 Declaration of Helsinki (World Medical Association of Helsinki 1997).

Of these 61 patients, 60 completed and returned the initial set of study questionnaires, making them eligible for the second phase of the study, i.e., neuropsychological testing, during their next clinic visit. Cirrhosis was histologically diagnosed in 44 patients and on the basis of clinical, radiological findings and ultrasonographic findings (reduced dimensions of the liver as well as splenomegaly and oesophageal varices observed by endoscopy), in the remaining 16 patients, in whom biopsy was contraindicated by uncontrolled coagulopathy and or uncontrolled ascites. Patients with a history of recent alcohol abuse, patients using psychotropic drugs (e.g., antipsychotics, interferon, benzodiazepines, anti-epileptics, sedatives and antidepressants) were excluded. Patients with fever, sepsis or shock were also excluded to avoid variations caused by body temperature. None of the patients had had a previous episode of spontaneous portal-systemic encephalopathy or chronic changes in mental state, and none was on treatment with interferon. Other 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) patients with metabolic disorders such as diabetes mellitus, unbalanced heart failure and/or respiratory failure or end-stage renal disease; (4) any additional precipitating factors such as high protein intake (additional high-protein meals), constipation; (5) illiteracy.

Eligible patients were randomly assigned to 1 of the 2 study treatments in equal proportions by means of a

computer-generated table of random numbers allocated in our central unit. They were divided into 2 groups (A and B)

Study design

Patients meeting inclusion criteria were randomized either into the group receiving a 90 days treatment with ALC (2 g twice daily) or into the group receiving placebo in double-blind. Patients were visited throughout the treatment period for assessment of adherence to the study protocol, blood pressure and cognitive function, as well as recording of adverse events. During the initial 2-week phase, subjects were instructed by a dietician to follow an "ad libitum" diet as follows: total fat 25–30%, saturated less than 7%, polyunsaturated up to 10%, monounsaturated up to 20%, carbohydrate 50–60% of total calories, proteins approximately 15%, cholesterol less than 200 mg per day (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001). Subjects were required to document all caloric intakes using a diary, to be completed thrice a week. This pre-randomization period was designed to nullify the effects of dietary changes on metabolic parameters. All administered drugs were identical in appearance, and neither investigators nor patients were informed of the selected agents at the end of the study. Administration instructions were provided with each patient pack. All patients were instructed to take the trial medication as prescribed. Subjects- compliance below 80% were excluded. Concomitant medications that the patients were receiving and that were continued throughout the study included neomycin, lactulose, lactitol, branched-chain amino-acids at the same dosage administered at enrolment.

Methods

Clinical, laboratory assessment, psychometric tests and automated EEG analysis were performed for all the patients. A detailed clinical neurological examination was performed. Selection of neuropsychological tests was based primarily on the necessity for assessment of relevant cognitive functions in a short period of time. Considering that the most consistently reported cognitive impairments in cirrhotic patients have been attention problems and psychomotor dysfunctions, the test battery used aimed to detect these problems. Assessment of learning and memory was also deemed important given the potentially adverse impact of these on daily functions. Additional criteria for inclusion in the test battery were: good psychometric properties, brevity and ease of scoring and administration and sensitivity to the effects of brain dysfunction. All measures were administered and scored according to standardized instructions.

Neuropsychological assessment

Trail Making Test (TMT)

This test was used to evaluate abstract reasoning, tactile performance, tactile-visual and spatial memory, rhythm perception and memory, speech-sound perception, primary motor speed, intelligence, psychomotor speed, sequencing abilities, language function, sensory function, grip strength and personality functioning. The TMTs are part of the Halsted-Reitan test battery (Reitan and Wolfson 1993). Time was recorded in seconds. This test included parts A and B. In part A, patients were asked to serially connect digits that were scattered on a page as quickly as possible. In part B, patients were asked to sequentially alternate numbers and letters (i.e., 1-A-2-B-3-C) as quickly as possible. A decrease in the time indicated an improvement in neuropsychological function. The score on each part represents the amount of time required to complete the task.

Mini Mental State Examination (MMSE)

The MMSE score ranges between 0 and 30. Test administration detects the following parameters: space time cognition (0–10), recent memory (0–3), attention and computing ability (0–5), recall (0–3) and language (0–9). This test may be applied in different linguistic areas without changes of its significance. The MMSE is used as a bedside screen for cognitive dysfunction (Folstein et al. 1975). A decrease indicates a worse performance.

Digit cancellation

This task consists of an $8_{1/2} \times 11$ in. page with 28 rows of 36 digits each. The patient is asked to cross out all of the 3 s as quickly as possible. The total time taken (in seconds) (DCT) and the number of errors, of omission and commission are recorded (DCE). Digit Cancellation is considered a test of sustained attention and concentration (Franklin et al. 1988).

Controlled Oral Word Association Test (COWAT)

COWAT is a language and executive function test that consists of three-phonemic-letter naming trials. The examiner asks the subject to say as many words as they can think in 1 min. beginning with a given letter (F, A, S). The score is the sum of all acceptable words (Benton 1994).

Judgement of line orientation (JLO)

A visuo-perceptual organization test that examines the ability between line segments forming a semicircle. The

score refers to the number of line pairs correctly matched. The total number of items is 30 (maximum score).

Logical Memory (Paragraph Recall)

Participants are required to repeat a story read aloud to them. Immediate recall was scored using a verbatim scoring procedure. This test measures short-term semantic memory (score 0–94) (Wechsler 1945).

Everyday Memory Questionnaire (EMQ)

This is a valid and reliable self-report measure of common memory lapses in everyday activities (Sunderland et al. 1983) comprising of 27 statements. Participants respond on a nine-point scale ranging from 'Not at all in the last 6 months' to 'More than once a day'. There are no sub-scales within this questionnaire. The higher the score the more forgetting is evident. Statements include "telling someone a story or joke that you have told them once already" and "forgetting where things are normally kept or looking in the wrong place for them" (score 0–224).

Hooper visual organization test

Hooper Visual Organization Test is a visual and executive test of perceptual organization that consists of a series of pictures of more or less readily recognizable cut-up objects which should be identified by the subject. The total number of items is 30 (maximum score) (Hooper 1983).

Neurophysiologic assessment

The EEG was recorded using standardized techniques. Five electrodes were attached to the skin at the position T3, T4, O1, O2, and Cz according to the international '10–20 system'. Electrode impedance was kept lower than 5 kO. After applying the usual bandpass filters (0.35–35 Hz), two runs of 100 s each were recorded and compared for reproducibility (Van Der Rijt and Schalm 1985). EEG tracking was performed before treatment and after 90 days of treatment. Modifications in EEG trackings were observed by distinct observer blindly and independently. EEG grading of HE was as follows:

Grade 0: HE was defined as the presence of a background activity (α rhythm).
Grade 1: a α rhythm with some scattered θ waves.
Grade 2: background activity of θ rhythm mixed with some δ and α waves.
Grade 3: background of polymorphic δ activity characterized by high amplitude with spontaneous variability.
Grade 4: δ activity characterized by small amplitude.

The mean cycle frequency of EEG was as follows:

Grade 0: normal a rhythm, 8–12 counts per second (cps)
Grade 1: 7–8 cps
Grade 2: 5–7 cps
Grade 3: 3–5 cps
Grade 4: <3 cps.

Liver function assessment

The Child-Pugh score was determined to assess the severity of cirrhosis, including three biochemical variables (serum albumin, bilirubin and prothrombin time) and two clinical characteristics (presence or absence of ascites and clinical HE). A patient has a Child-Pugh score A cirrhosis if the score is ≤6 points, Child-Pugh B if it is 7–9 points and Child- Pugh C if the score is >9 points. Patients without signs of ascites scored 2 points for ascites in Child-Pugh score (Pugh et al. 1973). We also evaluated the presence and severity of the porto-systemic shunt by the portal vein flow, by the presence and size of oesophageal varices and by splenic size.

Venous ammonia concentration

The ammonia determination was performed according to the enzymatic determination of ammonia with glutamate dehydrogenase in a rapid and interference-free photometric determination (340 nm) of NH_4^+ in native blood plasma according to Da Fonseca-Wollheim method (Da Fonseca-Wollheim 1973). Due to reasons of safety, blood after withdrawal was immediately taken by refrigerated transport to the laboratory for immediate (within 15 min from blood withdrawal) determination of NH_4^+.

Safety parameters

Safety parameters included blood tests (haemoglobin, haematocrit, white blood cell count, and thrombocytes) and liver function tests (alanine amino transferase, aspartate amino transferase, gamma glutammyl-transpeptidase, cholin-esterase activity, serum bilirubin concentrations, prothrombin time and partial thromboplastin time) on days 0, 30, 60 and 90.

Statistical analysis

Descriptive statistics were proposed from the study sample, and results were expressed as mean±SD. Statistical analyses were performed by two-way analysis of variance (ANOVA). All *P* values were two-sided, using a=0.05 as the reference standard for determining the significance of the principal outcomes. Statistical Analysis System (Cary,

NC) software version 6.11 was used for all analyses. The primary population for statistical analysis was the intent-to-treat population of all randomized patients (I.T.T.). To test the hypothesis that mean difference between groups was 20% against the hypothesis of no difference, with 90% power in a test with a two-sided 5% significance level, the required number of patients per group was estimated as $n>19$.

Results

Baseline values

Clinical characteristics of patients at randomization in both groups are presented in Table 2. The two groups were homogeneous for demographic characteristic, aetiology, casting of disease and Child-Pugh grade. Serum NH4+ fasting concentrations were not significantly different before the treatment. No statistical differences were observed between the two groups about prothrombin time and serum albumin, bilirubin, aspartate aminotransferase and alanine aminotransferase. No statistical differences have been observed in the two groups in the administered neuropsychological test and in EEG (Tables 3 and 4).

Neuropsychological response

At the end of treatment in the ALC group we observed significant differences in EMQ ($p<0.001$), LogR ($p<0.001$), TMT-A ($p<0.001$), TMT-B ($p<0.001$), COWAT ($p<0.001$), Hooper ($p<0.001$), JLO ($p<0.001$), DCT ($p<0.001$) and DCE ($p<0.001$).

When comparing the two groups we observed a significant difference in EMQ −23.9 vs 4.4 ($p<0.001$), LogR 22.3 vs 0.7 ($p<0.001$), TMT-A −7.5 vs −2.6 ($p<0.001$), TMT-B −10.5 vs −3.1 ($p<0.001$), COWAT 4.2 vs 0.5 ($p<0.001$), Hooper 2.6 vs 0.1 ($p<0.05$), JLO 2.8 vs 0.3 ($p<0.001$) and DCT −24.5 vs −2.4 ($p<0.001$) (Table 4).

Table 2 Baseline data of patients

Parameters	Group A ALC	Group B placebo
Male/Female	14/16	15/15
Age (range)	37–64	35–65
SBP (mmHg)	138±12	140±12
DBP (mmHg)	87±9	85±10
HF (bpm)	74±10	78±11
Cirrhosis aetiology		
Post-hepatitis B	7	6
Post-hepatitis C	12	11
Alcoholism	4	5
Cryptogenetic	7	8
Child-Pugh Class		
A	6	6
B	7	8
C	17	16

There were not significant differences between groups

SBP systolic blood pressure; *DBP* diastolic blood pressure; *HF* heart frequency; *bpm* beats per minute

Neurophysiologic response

At the end of the study period, 88% of patients treated with ALC and 72% of patients treated with placebo showed a significant improvement in EEG. The mean cycle frequency improved in 74% of patients treated with ALC and in 64% of patients treated with placebo (Table 3).

Biochemical responses

Effects of ALC on ammonia

At the end of treatment in the group treated with ALC we observed significant differences in NH_4^+ ($p<0.001$). In the comparison between groups there were significant differences in NH_4^+ 30.5 vs 13.5 ($p<0.001$) (Table 4).

Effects of ALC on liver function

At the end of treatment in the group treated with ALC we observed significant differences in AST ($p<0.001$) and ALT ($p<0.05$). In the comparison between groups there were significant differences in prothrombin time 2 vs 2.4 ($p<0.05$), ALT −10.7 vs −13.6 ($p<0.001$) (Table 4).

L-Carnitine in plasma and urine

In the ALC group, significant differences were observed in the following markers after treatment compared with baseline: free plasma carnitine (2.3 μmol/L, $P<0.001$), plasma concentrations of total plasma carnitine (3 μmol/L, $P<0.001$), plasma long-chain acylcarnitine (LCAC) (0.3 μmol/L, $P<0.001$), and short-chain acylcarnitine (SCAC) (0.5 μmol/L, $P<0.05$). No significant differences of levocarnitine concentrations were observed in the urine. In the placebo group the plasma concentrations of free L-carnitine and LCAC and the urinary excretion of free L-carnitine and SCAC did not show significant differences compared with baseline. At the end of the study period, compared with placebo, the ALC-treated patients showed significant improvements in the following markers: free plasma carnitine (2.3 compared with 0.1 μmol/L, $P<0.001$)

Table 3 E.E.G. grading in both groups before and after treatment

	ALC Group A		Placebo Group B	
Grade	Before treatment	After treatment	Before treatment	After treatment
0	0	5	0	1
1	0	8	0	8
2	5	10	5	10
3	20	6	22	10
4	5	1	3	1
Grade		MEAN CYCLE FREQUENCY		
0	0	3	0	1
1	0	5	0	3
2	13	12	8	11
3	15	10	20	14
4	2	0	2	1

plasma concentrations of total L-carnitine (3 compared with 0.4 μmol/L, $P<0.001$), plasma SCAC (0.5 compared with 0.2 μmol/L, $P<0.05$), plasma LCAC (0.3 compared with 0.1 μmol/L, $P<0.001$) (Table 5).

Discussion

We observed a significant improvement in neuropsychological response in patients with severe HE treated with

Table 4 Comparison between treatment groups

	Group A ALC		Group B Placebo	
	Before treatment	After treatment	Before treatment	After treatment
EMQ	157.6±18.7	133.7±13.7***^{A}	152.7±12	157.1±14*^{A}
MMSE	20.9±2	23.37±1.74*^{C}	21.6±1.7	22±1.6 *^{C}
LogR	41.9±10.5	64.2±9.6***^{A}	47.1±10.1	47.8±9.2* ^{A}
TMT-A	58.9±3.6	51.4±3.2*** ^{A}	59.8±5.1	57.2±4.8* ^{A}
TMT-B	69.4±4.3	58.9±6.6*** ^{A}	66.1±3.7	63±3.7* ^{A}
COWAT	22.4±.3	26.6±2.6*** ^{A}	22.1±2.6	22.6±2* ^{A}
Hooper test	21.3±2.8	23.9±1.5*** ^{B}	22.4±2.7	22.5±2.2* ^{B}
JLO	20.8±1.7	23.6±1.4*** ^{A}	21.8±2.1	22.1±1.4* ^{A}
DCT	200.6±12.7	176.1±13.1*** ^{A}	192±8.6	189.6±5.4* ^{A}
DCE	11.9±2.8	9.4±1.4*** ^{C}	8.7±2.3	9±1.6* ^{C}
NH_4^+(mg/dl)	114.3±14.4	83.8±16.8***^{A}	111.1±15.2	97.6±9.9 *^{A}
Albumin (g/dl)	3.3±0.4	3.5±0.4*^{C}	3.4±0.4	3.4±0.3 *^{C}
PT (%)	62.8±5.6	64.8±4.4*^{B}	59±5.8	61.4±6.3* ^{B}
Bilirubin (mg/dl)	2.1±0.6	1.8±0.6*^{C}	2.1±0.5	1.9±0.4 *^{C}
AST (IU/l)	119.2±13.1	102.2±12.6***^{C}	114.2±24.5	104.8±20.4* ^{C}
ALT(IU/l)	106.7±15.7	96±15**^{A}	136.3±31	122.7±19.4 *^{A}

EMQ Everyday Memory Questionnaire; *MMSE* Mini Mental State Examination; *LogR* Logical Memory (Paragraph recall) test; *TMT* Trail Making Test; *COWAT* Controlled Oral Word Association Test; *JLO* Judgement of line orientation; *DCT* Digit Cancellation time; *DCE* Digit Cancellation errors; *PT* prothrombin time; *AST* aspartate transaminase; *ALT* alanine transaminase

All values are expressed as mean±SD

Comparison within group A and within group B according to the values before the treatment

* P=NS; ** $P<0.05$; *** $P<0.001$

Comparison between groups A and B after treatment

^{A} $P<0.001$; ^{B} $P<0.05$; ^{C} NS

Table 5 Comparison of plasma and urinary concentrations of L-carnitine between treatment groups

	Group A ALC		Group B Placebo	
	Before treatment	After treatment	Before treatment	After treatment
Free plasma carnitine (μmol/L)	22±1.4	24.3±1.1***^{A}	22.9±0.8	23.0±0.8*^{A}
Plasma SCAC (μmol/L)	5.2±0.6	5.7±0.3**^{B}	5.2±0.5	5.4±0.4*^{B}
Plasma LCAC (μmol/L)	1.6±0.3	1.9±0.2***^{A}	1.4±0.2	1.5±0.2*^{A}
Total plasma carnitine (μmol/L)	28.9±1.7	31.9±1.2***^{A}	29.9±0.8	12±0.6*^{A}
Free urinary carnitine (μmol/L)	10.8±0.4	11±0.4*^{A}	10.2±0.3	10.2±0.3*^{A}
Urinary SCAC (μmol/L)	10.7±0.6	10.8±0.6*^{C}	10.7±0.3	10.7±0.3*^{C}

SCAC short-chain acylcarnitine; *LCAC* long-chain acylcarnitine

All values are expressed as mean±SD

Comparison within group A and within group B according to the values before the treatment

* P=NS; ** $P<0.05$; *** $P<0.001$

Comparison between groups A and B after treatment

^{A} $P<0.001$; ^{B} $P<0.05$; ^{C} NS

ALC. Results of this study revealed that patients with severe HE treated with ALC showed a decrease of cognitive deficits and an improvement in the domains of attention, learning, psychomotor speed, visuoconstructional skills and the ability to remember previously learned information. The pattern of cognitive dysfunction in HE is similar to that reported in patient with neurocognitive disorder associated with illness related dementia. HE in chronic liver failure is neuropathologically characterized by alterations of astrocyte morphology and function. Astrocytic swelling may occur but is generally insufficient to cause alterations in intracranial pressure. The characteristic morphologic change encountered in chronic liver failure is known as Alzheimer type II astrocytosis in which astrocytes exhibit a large swallen nucleus, prominent nucleolus and margination of the chromatin pattern (Butterworth et al. 1987; Neary et al. 1987; Butterworth 2002). Alzheimer type II cells also manifest alterations in expression of key astrocytic proteins, including glial fibrillary acidic protein, glutamate transporters and "peripheral type" (mitochondrial benzodiazepine receptors). Alzheimer type II astrocytes are also encountered in the brains of patients with chronic hyperammonemia due to inherited urea cycle disorders (Harper and Butterworth 1997) as well as in the brains of mice with urease-induced hyperammonemia and in cultured astrocytes exposed chronically to ammonia (Gregorios et al. 1985). Exposure of cultured astrocytes to ammonia also results in alteration of expression of glial fibrillary acidic protein, glutamate transporters and "peripheral-type" mitochondrial benzodiazepine receptors (Bélanger et al. 2002; Desjardins et al. 1999) similar to those reported in brain in chronic liver failure. ALC was originally considered of potential use in AD, because it can serve as precursor of acetylcholine. ALC appears to exhibit a significantly slower decline in some cognitive (Brooks et al. 1998; Thal et al. 2000). ALC administration has been reported to improve cognitive function in patients with AD and mood state in patients with senile depression (Pettegrew et al. 2000). Some studies have observed significant improvements in biochemical assay and psychometric tests in patients with AD treated with ALC (Montgomery et al. 2003). In addition ALC modulates phospholipids' metabolism, affects synaptic morphology and transmission of multiple neurotransmitters (Pettegrew et al. 2000) and protects against neurotoxicity evoked by mitochondrial uncoupling (Virmani and Binienda 2004). In ALC treated group we observed a significant decrease of ammonia. Administration of L-carnitine or ALC protects against ammonia toxicity (Matsuoka and Igisu 1993) restores high energy phosphate and acetyl-CoA levels and reinstates the compromised election transport chain in brains of experimental animals in chronic hyperammonemia (Ratnakumari et al. 1993; Rao et al. 1997; Qureshi et al. 1998) and in HE (Malaguarnera et al. 2006). In addition, there is evidence to suggest that L-carnitine prevents glutamate-evoked excitotoxicity. This effect, mediated by activation of metabotropic glutamate receptors, (Felipo et al. 1994, 1998) supports the excitotoxicity properties of ammonia. Ammonia is normally detoxified in the astrocytes, leading to the accumulation of intracellular glutamine. Glutamine is a powerful osmotically active substance that attracts extracellular water inside the astrocytes, provoking astrocyte swelling. In chronic liver failure there is a slow increase in brain glutamine which is partially compensated by a decrease in other osmotically active substances, mainly brain myo-inositol (Jover et al. 2006; Wright and Jalan 2007). Low grade brain edema is a central severe point in the pathogenesis of the HE in chronic liver disease (Häussinger et al. 2000). Brain edema and astrocyte swelling provoked

by glutamine accumulation in astrocytes should be an osmotic intracellular edema. It is important to take into account the dynamic character of brain edema in pathological situations, with the probable implication of other factors, such as activation of inflammatory response, that may be also involved in the pathogenesis of brain edema, especially in patient with overt HE (Poveda et al. 2010). The role of these factors might be explanation for the existence of a vasogenic extracellular edema instead of the hypothetic intracellular osmotic edema predicted by the low grade astrocytic swelling theory. Other possible explanations for the presence of extracellular edema in chronic liver failure might be changes in membrane permeability with extracellular migration of macromolecules, increased blood brain barrier permeability, changes in astrocytic shape due to oxidative stress (Häussinger and Schliess 2008). Carnitine and ALC participate in cell volume and fluid balancing in all tissues that are affected by the tonicity (iso-, hyper-, hypotonicity) of the extracellular environment (Peluso et al. 2000). Data suggest that despite fluctuations in carnitine concentration due to its osmolytic pressure changes, carnitine maintains its energy production capacities and often osmolytic gradients can be harnessed for energy (Peluso et al. 2000; Flanagan et al. 2010). The common underlying process in neurodegenerative processes is the increased metabolic stress due to mitochondrial dysfunction and formation of reactive oxygen species (ROS). This process has been linked to neurodegenerative disorders such as AD (Beal 1993; Hinerfeld et al. 2004). Positive effects of ALC supplementation on oxidative stress and cognition have also been reported. Feeding ALC to older rats lowered production of radical oxygen species, decreased oxidation of neuronal RNA and mutagenic aldehydes and cognition (Hagen et al. 2002). The antioxidant and energy-enhancing properties of ALC provide protection against neurotoxic agents (Binienda 2003). Attention, concentration abilities, problems with learning, psychomotor speed and mental flexibility appear to be affected earliest in HE. These deficits regardless of their cause may affect quality of life, performance in the work and home environment (Malaguarnera et al. 2011a, b). ALC treatment could be critical in diminishing detrimental effects on brain function in severe HE. The improvement of cognitive deficits, the reduction of ammonia, the modification of EEG in the patients treated with ALC suggest that ALC could represent a new tool in the treatment of severe HE.

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