PhD School of Neuropharmacology

XXVI Cycle

ROLE OF ACETYL-L-CARNITINE IN HEPATIC ENCEPHALOPATHY

Doctorate Thesis

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List of abbreviations

HE = Hepatic Encephalopathy
MHE = Minimal Hepatic Encephalopathy
ALF = Acute liver failure
PCS = Portacaval Shunt
CBF = Cerebral Blood Flow
BBB =Bloood Brain Barrier
EEG = Electro Encephalogram
GS = glutamine synthetase
mGluR = metabotropic glutamate receptor
NAc = the nucleus accumbens
MDT = mediodorsal thalamus
VMT = ventromedial thalamus
ATP = Adenosine Triphosphate

ICP = intracranial pressure

CSF = cerebrospinal fluid

BCAA = branched chain amino acids

Preface

Hepatic encephalopathy (HE) is a debilitating complication of cirrhosis which presents as a spectrum of neurological and neuropsychiatric dysfunction, affecting the patients consciousness, intellect, personality and neuromuscular activity.

Hepatic encephalopathy as a complication of cirrhosis leads to physical complication that affect function and performance of daily life such as fatigue, muscle cramps and asterixis, to neuropsychological complication such as shortened attention, disorientation in time or space, changes in personality and inappropriate behavior.

Understanding the various factor that affect the patient's quality of life depends in our realization of the multifaceted issues of cirrhosis and their effects on health status

DESIGN OF PRESENT RESEARCH

Design of the present research

The present thesis has focused on:

1) Elucidate pathophysiological mechanism of HE to date
2) The role of Acetyl-L-Carnitine
3) Examine the role of Acetyl-L-Carnitine in neurocognitive symptoms of HE
4) Examine the role of Acetyl-L-Carnitine in fatigue symptoms of HE

Chapter One

INTRODUCTION

Epidemiology

When the liver fails, owing to acute liver failure (ALF) or chronic liver disease such as chronic hepatitis or cirrhosis, the normal detoxification of endogenous and exogen compound is compromised and those compounds may reach the brain and affect cerebral function (Felipo 2013). (Felipo 2013) Although the incidence of ALF is low (around 2,000 people per year in the United States or Europe), mortality rates remain high. By contrast, chronic liver diseases affect 5.5 million individuals in the United States alone.

(Rose 2012) MHE (Covert Hepatic Encephalopathy) is characterized by decreased attention, poor concentration, impaired memory, sleep disturbances, reduced speed of information processing, and altered motor abilities. In addition, the subclinical cognitive impairment that characterizes MHE increases the risk of having a car accident. As such, MHE has a significant impact on patients' health-related quality of life and their ability to carry out day-to-day functions.(Stewart el al. 2007) As many as 80% (20–80%, depending on the severity of the disease) of patients with chronic liver disease may have MHE. Its presence also identifies patients with a fourfold higher risk of developing OHE. (Poordad, et al. 2007)

(Rosso Stepanova 2010) Overt HE occurs in approximately 30%– 45% of patients with cirrhosis, while minimal HE may

affect up to 60% of patients with chronic liver disease and up to 80% with cirrhosis. (Stepanova, et al. 2010)

The definitive data on the true incidence and prevalence of HE is lacking, mainly because of large differences in the etiology and severity of HE and relevant issues in diagnosing minimal HE (Lewis M and Howdle PD 2003; Poordad, et al. 2007; Randolph et al. 2009). Development of HE is associated with a poor prognosis. Specifically, in the presence of chronic liver disease, HE typically heralds hepatic decompensation, and its development is usually associated with high mortality, indicating the need for liver transplantation. (Benhaddouch Z, et al. 2007 ; Udayakumar N, et al. 2007 ; Findlay JY, et al. 2011; Bustamante J, et al. 1999).

Classification

Patients with clinical HE are classified into four grades. Patients in grade 1 show a trivial lack of awareness; experience euphoria or anxiety; have a shortened attention span; and have impaired performance in basic arithmetic, such as addition or subtraction. In grade 2, symptoms include lethargy or apathy; minimal disorientation in time or space; subtle changes in personality; and inappropriate behaviour. Patients in grade 3 experience somnolence to semistupor but remain responsive to verbal stimuli. They also experience confusion, gross disorientation and

exhibit bizarre behaviour. Patients in grade 4 are comatose. (Conn HO, et al. 1977)

In the last years, HE has been classified in covert and overt, the first one does not present clinical signs of the overt HE. Covert HE shows mild cognitive impairment, attention deficits, psychomotor slowing and impaired visuomotor and bimanual coordination. (Felipo V, 2013)

Pathophysiology

HE is characterized by neurocognitive and neuromotor impairment, this pathology measibly affect the quality of life of HE patients.

There is a general consensus that indicate ammonia and inflammation as a major characters in HE pathophisiology.

The two main underlying factors are **hyperammonaemia** and **inflammation**. The only mechanism for ammonia detoxification in the brain involves its incorporation into glutamine by glutamine synthetase, an enzyme that is present in the brain only in astrocytes. It has been proposed that glutamine accumulation in astrocytes owing to ammonia detoxification results in water entry and increased osmotic forces, which leads to astrocyte swelling and cytotoxic oedema. Glutamine-induced astrocyte swelling was considered to be a main mediator in all types of HE (Felipo V 2013).

Ammonia

Ammonia by-product of nitrogen metabolism, is produced mainly within the gut through the deamination of glutamine by glutaminase in the enterocytes of the small intestine and colon, as well as through the hydrolysis of urea, catalyzed by urease-producing bacteria that exist abundantly in the human gut. Gut-derived ammonia is transported and absorbed across the mucosal epithelium into the hepatic portal circulation, from which, in the case of a healthy liver, it is removed primarily through the urea cycle. This low-affinity, high-capacity ammonia detoxication system is present in the periportal hepatocytes located around the portal vein. Glutamine synthetase, another important ammonia-removing pathway located in the liver, catalyzes the conversion of glutamate into glutamine, thereby removing an ammonia molecule. This high-affinity, low-capacity reaction takes place in the perivenous hepatocytes located around the hepatic vein and acts as a scavenger for the ammonia that escapes periportal urea synthesis. The production of ammonia within the gut and its detoxication by the liver are the main pathways through which ammonia homeostasis is maintained in the body. However, other organs also contribute to ammonia metabolism. In addition to the liver and intestines, glutamine synthetase is found in the muscles and the brain (particularly in the astrocytes) and in phosphate-activated glutaminase in the kidneys and the brain (primarily in the neurons). In the presence of a healthy liver, blood ammonia levels are maintained in the low range of 35–60 μmol/l (Figure 2). However, during liver disease, given the reduced hepatic capacity for ammonia removal, the extrahepatic interorgan

ammonia metabolism is altered (including glutamine metabolism) (Wright G , et al. 2011), thus upsetting the balance between ammonia-producing/removing organs and ammonia homeostasis (Figure 2) (Rose 2012) However, other organs also contribute to ammonia metabolism. In addition to the liver and intestines, glutamine synthetase is found in the muscles and the brain (particularly in the astrocytes) and in phosphate-activated glutaminase in the kidneys and the brain (primarily in the neurons).. This results in a two to fivefold increase in blood ammonia, leading to an increase in ammonia levels in the brain, with deleterious consequences (Bosoi CR and Rose CF 2009 ; Felipo V and Butterworth RF 2002)

Inflammation

Neuroinflammation also contributes to encephalopathy and brain oedema in rats with ALF (Acute Liver Failure) (Jiang W et al., 2009) . The mechanisms and time course of these changes are different in various brain regions: ALF in rats increases cerebral blood flow (CBF) in the cortex but reduces it in the cerebellum. In patients with ALF, inflammation, cerebral oedema and increased CBF and lactate contribute to ICP and death (Jalan R, et al. 2004)

Hyperammonaemia per se induces neuroinflammation. Rats with chronic hyperammonaemia but without liver failure show microglial activation and neuroinflammation, especially in the cerebellum. Treatment of these rats with anti-inflammatory drugs restores cognitive function (Cauli O, et al. 2009) , which

suggests that hyperammonaemia-induced neuroinflammation has a major role in neurological impairment observed in MHE. In cultured microglia, ammonia upregulates the microglial activation marker allograft inflammatory factor 1 (also known as IBA1), which is also increased in the brain of patients with HE (Zemtsova I, et al. 2009) . Therefore, ammonia may act directly on microglia to induce their activation but could also induce neuroinflammation through peripheral effects that are then transduced to the brain. This may occur by transfer of pro-inflammatory cytokines from blood to the brain in the circumventricular organs in which the BBB is more permeable, or by direct infiltration of blood immune cells. Blood cytokines may also stimulate receptors on endothelial cells and trigger the release of inflammatory factors into the brain. Rats with MHE show neuroinflammation and cognitive and motor alterations that are reversed with anti-inflammatory drugs (Cauli O, et al. 2009; Cauli O, et al. 2007; Rodrigo R, et al. 2010). Inflammation exacerbates the cognitive deficits induced by hyperammonaemia (Marini JC and Broussard SR 2006), and hyperammonaemia has been shown to reduce motor coordination in rats with inflammation (Jover R, et al. 2006). The combination of hyperammonaemia and inflammation over a certain threshold induces mild cognitive impairment in humans even in the absence of liver disease (Felipo V, 2012). Together, the results from these studies suggest that targeting neuroinflammation may restore cognitive and motor function in patients with MHE (Felipo V, 2013).

Urea Cycle

(Felipo-Butterworth 2002) Hyperammonemia is associated with profound effects on Cerebral Brain Flood (CBF). These effects are dependent upon the severity and duration of hyperammonemia and show region selectivity. For example, in chronic mild hyperammonemia associated with liver cirrhosis, CBF is decreased in proportion to the deterioration of neuropsychiatric status (Posner JB and Plum F, 1960; James IM and Garassini M, 1971). Some studies demonstrated that there is a regional selectivity of the cerebral metabolic changes in hyperammonemia like a reduction in cortical structures and a concomitant increase in some sub-cortical areas (O'Carroll RE et al., 1991). Intracarotid infusions of ammonia sufficient to cause EEG slowing were found to result in increased cerebral metabolic rate for the glucose, which was confined to deep grey matter structures (Lockwood AH et al., 1982).

Since brain lacks carbamoyl-phosphate synthase I and ornithine transcarbamylase, it is unable to remove ammonia in the form of urea. Consequently, brain ammonia is metabolized almost exclusively to glutamine via the GS reaction. Glutamine synthesis remains the predominant route for ammonia removal in brain under both normal and hyperammonemic conditions (Cooper AJ and Plum P, 1987). In the brain, Glutamine synthetase is located only in astrocytes (Norenberg MD and Martinez-Hernandez A, 1979). Thus, it is the astrocyte rather

than the neuron that is uniquely responsible for ammonia detoxification in brain. Surprisingly, in contrast to peripheral tissue such as skeletal muscle, there is no significant induction of GS expression in brain in hyperammonemic states (Cooper AJ et al., 1985; Lavoie J et al., 1987). Moreover, , it is important to underline that since the enzyme functions at near maximal capacities under normal physiological conditions (Cooper AJ and Plum P, 1987).

Brain Energetic metabolism

Ammonia causes significant alterations of mitochondrial function and, consequently, changes in cerebral Energy metabolism. Ammonia stimulates glycolysis in brain extracts by activation of phosphofructokinase (Sugden PH and Newsholme EA, 1975) and acute ammonia toxicity in normal rats leads to brain ammonia concentrations in the 1.4–1.5 mM range resulting in increased brain glucose utilization (Hawkins RA et al., 1973). Increased brain glucose concentration in acute hyperammonemia may be the consequence of an increased in expression of the endothelial cell/astrocytic glucose transporter GLUT-1 as was recently reported in experimental ALF (Desjardins P et al., 2001). Increased brain glucose uptake was accompanied by increased brain lactate concentrations, which occurred without any loss of high Energy phosphates. The increased brain glucose uptake and lactate accumulation due to acute ammonia exposure appears to be predominantly an astrocytic phenomenon since expression of the neuronal glucose

transporter GLUT-3 is not affected by ammonia (Desjardins P et al., 2001).

During hyperammonaemia, it was also reported a reduction in ATP (McCandless DW and Schenker S, 1981; Kosenko E et al., 1994), but several groups have found but most of these articles show this reduction in animal models exposed to an hyperamonemia of 3 mM, similar or sometimes higher than an hepatic coma. Infact, there is little convincing evidence to suggest that hyperammonemia resulting from acute or chronic liver failure results in a loss of ATP in brain at least until stages of encephalopathy characterized by prolonged coma (Mans AM et al., 1994; Hindfelt B et al., 1977). Likewise, studies in cirrhotic patients with end-stage liver failure using spectroscopic techniques have so far not provided convincing evidence for a primary cerebral energy deficit (Taylor-Robinson SD et al., 1994; Lockwood A et al., 1997).

Two distinct mechanisms have been proposed to explain ammonia-induced reductions in brain ATP:

(1) inhibition of the tricarboxylic acid cycle (TCA);

(2) a mechanism involving N-methyl-d-aspartate (NMDA) receptors.

In favour of the first mechanism, McKhann and Tower (1961) reported ammonia-induced inhibition of the TCA cycle in brain with accumulation of alpha-ketoglutarate and pyruvate. Subsequently, Lai and Cooper (1986) described a significant inhibition of the rate-limiting TCA cycle enzyme alpha-ketoglutarate dehydrogenase (alpha-KGDH) in brain mitochondrial preparations exposed to ammonia with an EC50 of

2 mM. Consistent with alpha-KGDH inhibition and a consequent reduction in entry of pyruvate into the tricarboxylic acid cycle are the findings of increased brain lactate concentrations in various hyperammonemic disorders (Hawkins RA et al., 1973; Hindfelt B et al., 1977; McCandless DW and Schenker S, 1981; Therrien G et al., 1991; Mans AM et al., 1994; Chatauret N et al., 2001). Furthermore, hypoxia significantly exacerbates the effects of lethal injections of ammonium salts in mice (Warren and Schenker, 1960). Cerebrospinal fluid lactate concentrations are increased in both acute (Chatauret N et al., 2001) and chronic (Therrien G et al., 1991) liver failure and are positively correlated with the severity of HE in these disorders. Increased CSF lactate has also been reported in human HE (Yao H et al., 1987). According to this hypothesis involving inhibition of the TCA cycle by ammonia is the report that increased ammonia levels in animals injected with U 14C glucose resulted in a reduction in the amount of label incorporated into the amino acids glutamate and GABA (Prior RL and Visek VJ, 1972).

In support of a pathogenetic role of NMDA receptors, it has been shown that ammonia-induced depletion of brain ATP in vivo is prevented by administration of a wide range of glutamate (NMDA) receptor antagonists (Kosenko E et al., 1994). Based upon these observations, it was suggested that ammonia-induced activation of NMDA receptors results in ATP depletion via the activation of Na+, K+, ATPase as well as by decreased synthesis of ATP due to impairment of Ca2 + homeostasis (Kosenko E et al., 2000).

There are several possible explanations for the absence of cerebral energy deficit in chronic liver failure.

1) Proliferation of astrocytic mitochondria has been reported in conditions of chronic hyperammonemia (Gregorios JB et al., 1985; Norenberg MD and Lapham LW, 1974), a phenomenon that has been attributed to increased energy requirements.
2) It has been reported that chronic hyperammonemia similar in magnitude to that observed in end-stage chronic liver failure leads to down-regulation of functional NMDA receptors (Peterson C et al., 1990; Marcaida G et al., 1995).
3) The impairment of signal transduction pathways associated to NMDA receptors could determinate the lack of energy depletion in chronic hyperammonemia (Hermenegildo C et al., 1998).

Neurotrasmission

Alterations in glutamatergic and GABAergic neurotransmission in different brain regions contribute to altered motor function in MHE. The neuronal circuits between the basal ganglia, thalamus and cortex that modulate motor activity are altered in patients with MHE as well as in rats with hyperammonaemia and MHE, and lead to motor impairment, including hypokinesia (Cauli O, et al. 2006; Agusti A, et al. 2011). In vivo microdialysis studies show that in control rats, metabotropic glutamate receptor (mGluR) activation in the nucleus accumbens (NAc) increases

the level of extracellular dopamine, which activates dopamine receptors, inducing GABA release in the ventral pallidum and a reduction of GABA levels in the mediodorsal thalamus (MDT). In turn, this reduced inhibition by GABA leads to increased glutamate release in the cortex, resulting in increased motor activity. However, hyperammonaemia or MHE lead to the activation of an 'alternative' circuit involving the NAc, the SNr and the ventromedial thalamus (VMT). In rats with MHE, mGluR activation in the NAc does not increase extracellular dopamine levels but does increase glutamate levels, which activates AMPA receptors, inducing GABA release in the SNr and a reduction of GABA levels in the VMT. This reduced inhibition by GABA results in an increased level of extracellular glutamate in the cortex and enhances motor activity in rats with MHE but not in control rats (Cauli O, et al. 2006; 2007b).

Under normal conditions (without the exogenous activation of mGluRs in the NAc described above), rats with MHE show less spontaneous motor activity than normal rats and similar motor impairments to those observed in patients with HE (hypokinesia). This hypokinesia is due to increased extracellular glutamate levels in the substantia nigra pars reticulata (SNr) of rats with MHE, which results in excessive mGluR1 activation, which then increases extracellular GABA levels in the VMT and reduces extracellular glutamate levels in the cortex. Blocking mGluR1 by stereotaxic injection of the selective antagonist CPCCOEt in the SNr normalizes extracellular GABA levels in the VMT and glutamate levels in the cortex, and eliminates hypokinesia , supporting the idea that excessive levels of

extracellu-lar glutamate and activation of mGluR1 in the SNr are responsible for hypokinesia. A main contributor to increased extracellular glutamate levels in the SNr of MHE rats is the reduced amount and function of the glutamate transporters EAAC1 (also known as excitatory amino acid transporter 3) and GLT1 (also known as excitatory amino acid transporter 2) in the SNr. The amount of glutamate transporter is normalized and extracellular glutamate levels are reduced in rats with MHE by administration of an anti-inflammatory drug (ibuprofen) that reduces neuroinflammation and also eliminates hypokinesia. These findings demonstrate that, as mentioned above, both hyperammonaemia and neuroinflammation contribute to motor alterations in MHE. Although non-steroidal anti-inflammatory drugs such as ibuprofen are not recommended in cirrhotic patients, owing to risk of secondary effects on the kidneys, inhibitors of p38 also reduce neuroinflammation and eliminate hypokinesia in rats with MHE and may improve motor function in patients with MHE or clinical HE.

Activation of the glutamate ionotropic receptor N-methyl-D-aspartate (NMDA) has been demonstrated to play an important role in the pathophysiology of hepatic encephalopathy (Llansola M, et al. 2007). The opening of the channel is controlled by a powerful voltage-dependent block by external magnesium ions (Mayer ML, et al. 1984; Nowak L, et al. 1984). It is believed that ammonia, by raising the membrane potential,removes the magnesium block rendering NMDA receptors susceptible to activation. However the mechanism and the degree in which is

involved ammonia in the removing of the magnesium ion block in these receptors has not been clarified. Pathophysiological concentrations of ammonia directly raise the membrane potential in both astrocytes and neurons, however, not sufficiently to activate voltage-gated channels or generate an action potential in neurons (Bosoi CR and Rose CF 2009).

These mechanisms could effectively minimize the impact of mechanism involving N-methyl-d-aspartate (NMDA) receptors and prevent loss of ATP due to NMDA receptor activation. Consistent with these possibilities, it has been shown that chronic moderate hyperammonemia in rats completely prevents the depletion of ATP induced by subsequent acute lethal injections of ammonia (Kosenko E et al., 1993).

Studies in rats support the idea that cerebral damage in ALF involves an initial disruption of BBB permeability leading to vasogenic oedema in certain areas such as the cerebellum but not in the frontal cortex. At early stages of ALF, oedema is mainly vasogenic and is associated with increased intracranial pressure (ICP) (Cauli O, et al. 2011) , indicating that astrocyte swelling is not the initial trigger of oedema and ICP. As brain ammonia and glutamine progressively increase, cytotoxic oedema (which is probably due to astrocyte swelling) ensues in many areas, further increasing ICP.

At least 16 enzymatic pathways in brain result in the formation of ammonia. One of the most important is glutamate dehydrogenase, which catalyses the reversible oxidative deamination of glutamate. It has been proposed that both in normal and hyperammonemic conditions, glutamate

dehydrogenase is ammonia producing, particularly in astrocytes and, in this way, may provide a mechanism for the removal of excess nitrogen from certain catabolyzed amino acids (Cooper AJ and Plum P, 1987). A catabolic role for glutamate dehydrogenase is also consistent with the finding of decreased brain glutamate concentrations in a wide range of hyperammonemic syndromes (Lavoie J, et al., 1987; Swain M, et al. 1992; Ratnakumari L, et al. 1994). L-Glutaminase is widespread in brain and is particularly abundant in nerve endings of glutamatergic neurons where it forms an integral part of the glutamate-glutamine cycle in which a molecule of ammonia is transferred from the astrocyte to the neighbouring neuron. There is evidence to suggest that at least part of the increased glutamine encountered in brain in hyperammonemia results from inhibition of glutaminase (Tyce GM, et al. 1981). Enzymes of the purine nucleotide cycle may also be responsible for generating a significant fraction of brain ammonia (Schultz V and Lowenstein JM, 1978).

NMDA receptors modulate learning and memory, and the glutamate–nitric oxide–cGMP pathway has an important role in these processes. In rats with MHE, reduced functioning of this pathway in the hippocampus leads to impaired long-term potentiation (LTP) and spatial learning in the Morris water maze. Furthermore, reduced signalling through this pathway in the cerebellum has been reported in rats with hyperammonaemia or MHE in vivo and correlates with impaired learning of a conditional discrimination task in a Y maze. In patients that died for HE, lower cGMP formation in the cerebellum is thought to be

involved in their reduced learning ability. Restoration of extracellular cGMP levels in the cerebellum restores learning in rats, and this can be achieved by inhibiting phosphodiesterase 5. Studies on the mechanisms by which HE impairs signalling through the glutamate–nitric oxide–cGMP pathway have identified other modulators of the cGMP pathway that could be pharmacologically targeted to restore learning.Chronic hyperammonaemia has also been shown to increase tonic activation of NMDA receptors in the cerebellum, leading to the activation of calcium/calmodulin-dependent protein kinase II (CaMKII) and increased phosphorylation of neuronal nitric oxide synthase (NOS) on Ser847. This reduces the enzyme's activity and, thus, the formation of nitric oxide and cGMP. Chronic hyperammonaemia also leads to a subcellular redistribution of NOS, as reduced amounts reach synaptic membranes. As a result, activation in response to NMDA receptor activation is reduced, and nitric oxide and cGMP formation is further decreased. Increases in tonic activation of GABA A receptors in the cerebellum have also been reported, leading to reduced functioning of the pathway and cGMP formation. Blocking GABA A receptors with bicuculline restores signalling and learning in rats. Neuroinflammation has also been shown to mediate some of the effects of hyperammonaemia on the glutamate–nitric oxide–cGMP pathway. Treatment of MHE rats with anti-inflammatory drugs (ibuprofen) reduces microglial activation and neuroinflammation in the cerebellum and restores learning . Microglial activation and neuroinflammation could also be reduced with MAPK p38

inhibitors, which have been shown to restore learning ability in rats with HE.

Cerebral Oedema

A large amount of studies demonstrated that cerebral oedema is implicated in all of the forms of HE, but in most of these studies was used an high ammonia concentration that does not reflect the concentration found in chronic HE patients (Felipo 2013). In vivo data in patients with chronic liver disease do not support a role for astrocyte swelling in their HE. The findings from functional MRI (fMRI) studies in patients with chronic liver disease and HE suggest that they develop vasogenic oedema, as an increase in the apparent diffusion coefficient was detected rather than a decrease (which would be expected in cytotoxic oedema). Thus, cytotoxic oedema is unlikely to be the main cause of neurological alterations in MHE. Rats with MHE also show cognitive and motor alterations in the absence of oedema.

Other causes

in addition to ammonia, chronic liver failure results in the accumulation of other toxins including manganese (Pomier Layrargues G, et al. 1995), mercaptans and short-chain fatty acids (Zieve L, et al. 1974), all of which may have deleterious effects on brain function.

The role of Acetyl-L-Carnitine in the treatment of HE

L-Carnitine (LC), acylcarnitines, and various carnitine enzymes constitute the carnitine system that play a pivotal role in cellular energy production. The system is ubiquitous

and the mitochondrial carnitine system has an obligatory role in beta oxidation of long-chain fatty acids by their transport into the mitochondrial matrix. LC and its esters are present in different concentrations in human serum (L-carnitine/acetyl-L-carnitine / propionil-L-carnitine = 5:1:0,1). Carnitines are involved in the removal of accumulated toxic fatty acyl-CoA metabolites and helping in the balance between free and acyl-CoA. The toxic effects of poorly metabolized acetyl groups can be lowered with transesterification from CoA and excretion of ALC esters by carnitine acetyltransferase (CAT) and carnitine palmitoyltransferases (CPT-1 and CPT-2).

L-carnitine was first used in Reye Syndrome, a syndrome characterized by a deficiency of Carnitine. Reye syndrome is biochemically distinct from the clinically similar syndromes of systemic carnitine deficiency. In this syndrome patients show an impaired liver function and some signs similar to hepatic encephalopathy such as increased ammonia levels and a damaged brain function.

One of the first pathophysiological explanation proposed for hepatic encephalopathy was the altered brain metabolism caused by a decrease in ATP concentration due to a decreased ATP production because of non-optimal operation of the Krebs cycle and inhibition of the mechanism for introducing reducing equivalents into mitochondria (O'Connor JE, et al. 1984). On the basis of this evidence L-carnitine was tested in mice with an acute ammonia intoxication. L-carnitine showed decreased ammonia concentration in these mice finally increasing the

survival rate (O'Connor JE, et al. 1984). The possible explanation of the lowering ammonia concentration effect of L-Carnitine was also explained with the blocking of malate aspartate shuttle leading to increase ATP production via oxidative phosphorylation and also for the increase of Acetyl CoA due the avaibility of acetylic moieties for the increase of the beta-oxidation reaction in the mitochondria. Acetyl CoA should enhance the synthesis of N-acetylglutamate, the physiological activator of carbamyl phosphate synthetase I, and thus urea synthesis with a concomitant increase in ammonia utilization (O'Connor JE, et al. 1984).

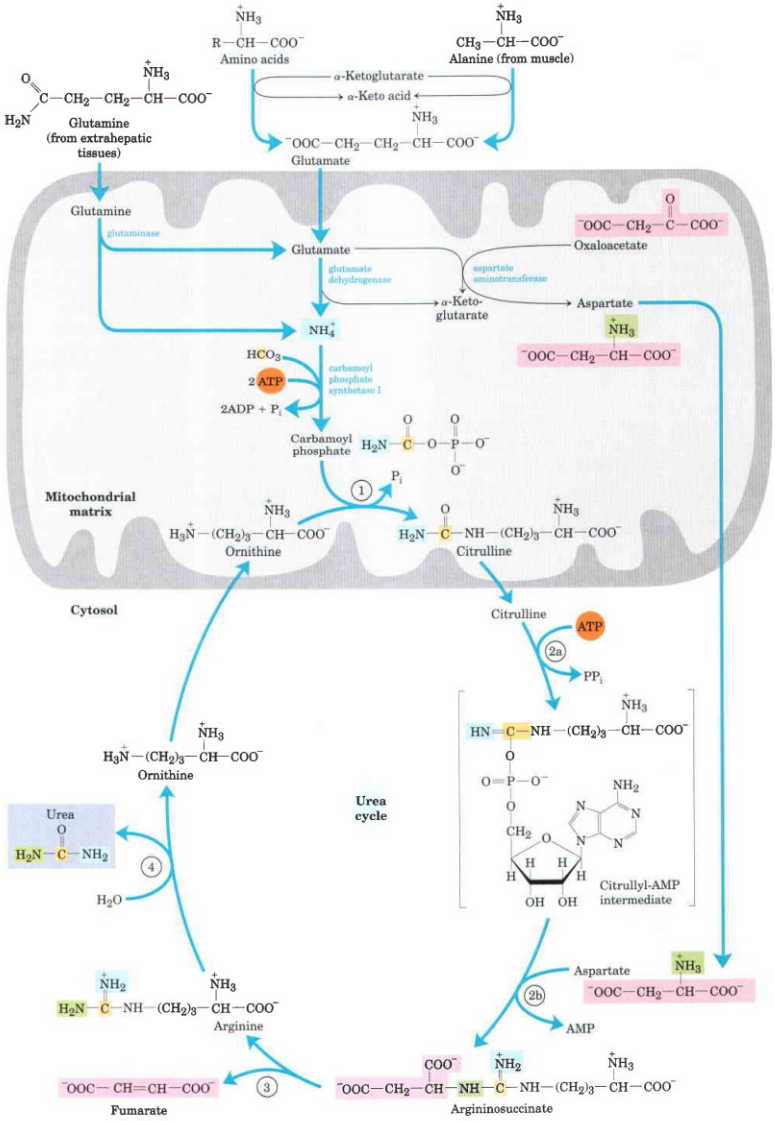

Figure 1. Urea cycle and reactions that feed amino groups into the cycle. The enzymes catalyzing these reactions (named in the text) are distributed between the mitochondrial matrix and the cytosol. One amino group enters the urea cycle as carbamoyl

phosphate, formed in the matrix; the other enters as aspartate, formed in the matrix by transamination of oxaloacetate and glutamate, catalyzed by aspartate amino-transferase. The urea cycle consists of four steps 1) Formation of citrulline from ornithine and carbamoyl phosphate (entry of the first amino group); the citrulline passes into the cytosol. 2) Formation of argininosuccinate through a citrullyl-AMP intermediate (entry of the second amino group). 3) Formation of arginine from argininosuccinate; this reaction releases fumarate, which enters the citric acid cycle. 4) Formation of urea; this reaction also regenerates ornithine (Lehninger)

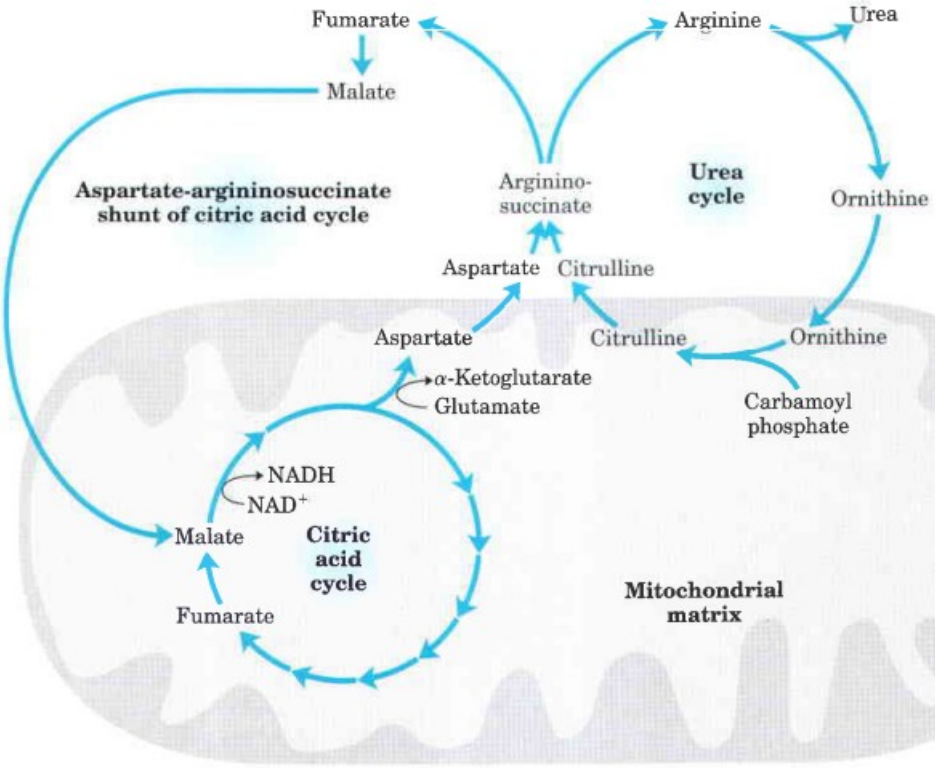

Figure 2. Links between the urea cycle and citric acid cycle. The interconnected cycles have been called the "Krebs bicycle" The pathways linking the citric acid and urea cycles are known as the aspartate-argininosuccinate shunt; these effectively link the fates of the amino groups and the carbon skeletons of amino acids. The interconnections are even more elaborate than the arrows suggest. (Lehninger)

L-Carnitine is acylated by L-carnitine acyltransferases (e.g. palmitoyltransferase) and it is transported into the mitochondrial matrix by L-carnitine translocases, which exchange L-carnitine with acyl-L-carnitine. In the mitochondrial matrix, acyl-L-carnitine is used to form acyl-CoA by acyltransferases (Fritz IB, 1959; Haeckel R et al., 1990).

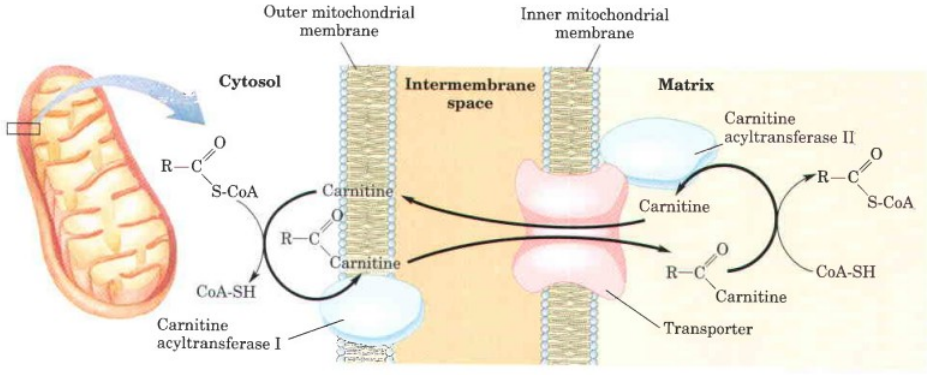

Figure 3. Fatty acid entry into mitochondria via the acyl-carnitine/carnitine transporter. After fatty acyl-carnrtine is formed at the outer membrane or in the intermembrane space, it moves into the matrix by facilitated diffusion through the transporter in the inner membrane. In the matrix, the acyl

group is transferred to mitochondriat coenzvme A, freeing carnitine to return to the intermembrane space through the same transport.

Primary genetic disorders of L-carnitine metabolism are due to inherited enzyme deficiencies, for example, carnitine palmitoyltransferase (CPT I or CPT II) deficiencies. Secondary deficiencies (reduction in plasma concentration) may be due to a number of conditions affecting intermediary metabolism: organic acidemias, inherited fatty acid oxidation disorders due to deficiencies in enzymes or proteins involved in mitochondrial beta-oxidation or respiration or in the urea cycle. Some nongenetic disorders also result in reduced plasma L-carnitine, for example, AIDS, chronic haemodialysis, or treatment with sodium valproate or with antibiotics that contain pivalic acid. In most cases carnitine deficiency is associated with hyperammonemia (Breningstall GN, 1990; Haeckel R, et al., 1990).

Reye's-like syndrome is also induced by valproate, an anti-epileptic drug that may cause hepatotoxicity, hyperammonemia, hypoketonemia, and a decrease of L-carnitine levels. L-Carnitine treatment of patients with valproate-induced hepatotoxicity restores plasma ammonia levels and improves hepatic function (Bohan TP, et al. 2001).

Many studies report a recovering of energy metabolism and urea cycle enzymes, in different animal models of hyperammonemia (Ratnakumari L, et al. 1993 ; Horiuchi M, et al. 1992 ; Hearn TJ, et al. 1989).

In PCS (Portocaval-shunt) rats, that is one of the main animal models used to study first grade of HE showed that L-Carnitine prevents increase of ammonia levels in cerebrospinal fluid (CFS) and normalizes levels of alanine and lactate in CFS (Therrien G, et al. 1997). Alanine and lactate used to increase during hepatic encephalopathy, as I previously showed, for an impairment in the aminoacid metabolism and for the increase of glycolitic pathway. Different studies have been carried out trying to unveil the mechanism of this protective effect of L-carnitine. Other quaternary amines (betaine, choline, or trimethylamine N-oxide) like L-carnitine also have a protective effect against ammonia toxicity and the authors suggested that osmoregulation is involved in the mechanism of this protective effect (Kloiber O, et al. 1988). The protection due the osmoregulation property of quarternary amines were confirmed using same compounds and others with similar chemistry property (trimethylamine N-oxide, choline, acetylcholine, carbachol, and acetyl-L-carnitine). At low concentration these quaternary amines showed a protection at low concentration during ammonia toxicity in mice (Miñana 1996)

Dr. Felipo's group from the center of investigation "Principe Felipe" of Valencia, have studied the neurobiology and possible treatment of HE. In the 1992 they demonstrated that acute ammonia toxicity in the brain is mediated by excessive activation of NMDA glutamate receptors (Marcaida et al., 1992). Ten different antagonists of NMDA receptors acting on three different sites of the receptor prevent ammonia toxicity in rats and mice injected with lethal doses of ammonia acetate

(Hermenegildo et al., 1996). They studied whether prevention of ammonia toxicity by L-carnitine is due to prevention of glutamate neurotoxicity, using primary cultures of cerebellar neurons to study the effect of L-carnitine on glutamate neurotoxicity. Treatment of these neurons in culture with 1 mM glutamate causes death of 80% of neurons. In this system glutamate neurotoxicity is mainly mediated by activation of NMDA receptors. Addition of 1 mM L-carnitine 15 min before glutamate prevented neuronal death caused by glutamate. However the high concentration of carnitine required is in agreement with the large doses of L-carnitine necessary to completely prevent ammonia toxicity in animals. In the same study, they tested whether L-carnitine affects the binding of glutamate to its receptors in hippocampal rat membranes. L-Carnitine increased the affinity of [3 H]-glutamate binding to the receptors. This increase was due to an increase of binding affinity to “quisqualate” receptors, whereas the affinity for the binding to NMDA and kainate receptors was slightly decreased (Felipo et al., 1994). In 1994, a specific agonist for “quisqualate” receptors was not available. For this reason they used Quisqualate, an unspecificic agonist that activates AMPA and metabotropic glutamate receptors. This suggests that in the presence of L-carnitine, glutamate binding to metabotropic receptors is increased. They also assessed whether the increase in the binding affinity of metabotropic glutamate receptors induced by L-carnitine is involved in its protective effect against glutamate neurotoxicity. AP-3, an antagonist of metabotropic glutamate receptors prevented the protective effect of L-carnitine

against glutamate neurotoxicity. Moreover, pre-incubation with t-ACPD, an agonist of metabotropic glutamate receptors also prevented glutamate neurotoxicity (Felipo et al. 1994).

L-carnitine and trimethylamine-containing compounds do not prevent neurotoxicity induced by NMDA, this fact supports the idea that the protective effect of these compounds is mediated by an increase of glutamate binding to metabotropic glutamate receptors. The affinity of NMDA for metabotropic receptors is not significant and NMDA would not activate these receptors in the presence of either carnitine or the other protective compounds. Some of the trimethylamine-containing compounds (acetylcholine, carbachol) are agonists of acetylcholine receptors. Atropine, an antagonist of acetylcholine receptors, also prevents the protective effect of most of these compounds, including that of t-ACPD (agonist of metabotropic glutamate receptors), against glutamate neurotoxicity.

Injection of atropine also prevents the protective effect of some of the trimethylamine-containing compounds against ammonia toxicity in mice. The protective effect of L-carnitine and betaine is not prevented by atropine (Miñana et al., 1996). These results show that antagonists of both acetylcholine and metabotropic glutamate receptors prevent the protective effect of trimethylamine-containing compounds against glutamate neurotoxicity, suggesting that there is an interplay between both types of receptors in the protective effects of L-carnitine against glutamate neurotoxicity (Llansola M, et al. 2002)

Subsequently, the same group published a study demonstrating that the activation of the mGluR5 subtype is responsible for the

protective effect of metabotropic glutamate receptor agonists (Montoliu et al., 1997). Recently, it was assessed that LAC treatment in mice could increase mGluR2 expression but not mGluR3 levels in hippocampus (Cuccurazzo 2013), but no studies have been conducted to assess a particular role of these receptors during ammonia intoxication. According to the literature, activation of mGluR5 is associated with activation of phospholipase C. Phospholipase C hydrolyzes inositol phospholipids releasing inositol triphosphate (IP 3) and diacylglicerol (DAG). Inositol triphosphate induces release of calcium from internal organelles and DAG activates protein kinase C.

In hippocampal slices, addition of the metabotropic glutamate receptor agonist t-ACPD induces an increase in phospholipid hydrolysis. They expected that L-carnitine would induce an increase in t-ACPD-induced phospholipid hydrolysis, i.e., a greater activation of metabotropic glutamate receptors. However, pre-incubation with L-carnitine inhibited t-ACPD-induced hydrolysis of phospholipids in a dose-dependent manner. Moreover, L-carnitine also inhibited phosphoinositide hydrolysis induced by arterenol, an agonist of noradrenergic receptors, and partially inhibited the effect of carbachol, agonist of acetylcholine muscarinic receptors (Llansola and Felipo, 1998). These results suggest that L-carnitine affects phosphoinositide hydrolysis induced by different types of receptors. . The same group also examines whether L-carnitine affects G-protein affects G-protein function by assessing the effect of L-carnitine on phosphoinositide hydrolysis induced by AlF 4 ¡, that directly

activates G-proteins. L-Carnitine inhibited partially (45%) phosphoinositide hydrolysis induced by AlF 4-. This suggests that L-carnitine affects some types of heterotrimeric G-proteins (Llansola and Felipo, 1998).

CHAPTER I

Oral acetyl-L-carnitine therapy reduces fatigue in overt hepatic encephalopathy: a randomized, double-blind, placebo-controlled study*

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Abstract

Background: Fatigue is frequently reported in hepatic encephalopathy (HE) and may be related to hyperammonemia. Acetyl-L-carnitine (ALC) offers neuroprotective benefits and improves mitochondrial energetics and function.

Objective: This study evaluated the effect of exogenous ALC on physical and mental fatigue, fatigue severity, and physical activity in patients with mild and moderate hepatoencephalopathy (HE1 and HE2, respectively).

Design: A total of 121 patients with overt HE were recruited to the study and were subdivided into 2 groups according to their initial HE grade [HE1 (n = 61) or HE2 (n = 60)]. Thirty-one patients with HE1 and 30 with HE2 received 2 g ALC, and 30 patients with HE1 and 30 patients with HE2 received placebo twice a day for 90 d. All patients underwent clinical and laboratory assessments and automated electroencephalogram analysis.

Results: At the end of the study period, the ALC-treated patients in the HE1 group showed significantly better improvement than did the placebo group in mental fatigue score (−1.7 compared with −0.3; $P < 0.05$), the fatigue severity scale (−6.4 compared with 2.3; $P < 0.001$), 7-d Physical Activity Recall questionnaire score (17.1 compared with −2.5; $P < 0.001$), and Short Physical Performance Battery (2.1 compared with 0.2; $P < 0.001$); the HE2 group showed significantly better improvement in the fatigue severity scale (−8.1 compared with −5.1; $P < 0.001$) and 6-min walk test (19.9 compared with 2.3; $P < 0.05$). Significant decreases in NH_4^+ were observed in both groups ($P < 0.001$).

Conclusion: Patients with HE treated with ALC showed a decrease in the severity of both mental and physical fatigue and an increase in physical activity. This trial was registered at clinicaltrials.gov as NCT01223742.

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1. Introduction

Hepatic encephalopathy (HE) is a neuropsychiatric complication of cirrhosis. Overt HE can be diagnosed clinically, and a mild-to-moderate grade of disease might be present in a considerable proportion of ambulatory patients with cirrhosis. Overt HE is a syndrome of neurologic and neuropsychiatric abnormalities. Affected patients exhibit alterations in psychomotor, intellectual, cognitive, emotional, behavioral, and fine motor function. Fatigue is frequently reported in HE and can also be related to hyperammonemia (1). Ammonia is recognized as a crucial component in the pathogenesis of HE, but other factors, such as oxygen free radicals, circulating opioid peptides, nitric oxide, inflammatory cytokines, reduction in serotoninergic neurotransmitters, depletion of endogenous antioxidants, neurosteroids, and manganese, are also implicated in the development of the disease (2). In recent years, fatigue has been researched as the main symptom of elevated ammonia in HE (1, 3). The treatments that remove ammonia from the body or that decrease ammonia production and absorption through the gastrointestinal tract improve mental status and cognitive function, but no effects have yet been shown in fatigue treatment. Our previous study showed a protective effect of L-carnitine against ammonia-evoked encephalopathy in cirrhotic patients, and another study showed that acetyl-L-carnitine (ALC) administration improved neurologic symptoms and plasma variables in selected cirrhotic patients with hepatic coma. Finally, other studies showed that ALC treatment reduces fatigue

in the elderly and in centenarians (4–7). ALC is an endogenous molecule synthesized in mitochondria by the enzyme ALC transferase and is the predominant acylcarnitine in normal tissues. Acylcarnitine is the fatty acid–bound form of L-carnitine, which has an important role in the transport of long-chain fatty acids into mitochondria and in their β-oxidation (8–10). Serum acylcarnitine is mainly composed of short-chain fatty acid L-carnitine, especially ALC. Although 99% of the amount of L-carnitine is intracellular, the relation between serum acylcarnitine and free L-carnitine is highly sensitive to intramitochondrial metabolic alterations (11). ALC treatment restores the altered neurochemical abnormalities, cerebral energy metabolites in ischemia and aging and, in particular, ammonia-induced cerebral energy depletion (12). It also facilitates the removal from the mitochondria of excess short- and medium-chain fatty acids that accumulate during metabolism (13). Some of ALC's proposed neuroprotective benefits involve improved mitochondrial energetics and function, antioxidant activity, stabilization of membranes, protein and gene expression modulation, and enhancement of cholinergic neurotransmission (14). Patients with fatigue show reduced exercise tolerance and postexercise fatigue induced by minimal physical activity, which suggests decreased muscle function. During physical activity, the rate of free radical formation may overcome the various protective defense mechanisms and induce systemic oxidative stress through plasma accumulation of secondary products of lipid peroxidation (15). The aim of this study was to evaluate the effect of exogenous ALC on physical and mental fatigue, fatigue

severity, and physical activity in patients with mild and moderate encephalopathy.

2. Subjects and Methods

Subjects

A total of 121 cirrhotic patients (22 with hepatitis B virus infection, 65 with hepatitis C virus infection, 9 with alcoholism, and 25 with cryptogenetic cirrhosis) meeting the following inclusion criteria 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 a Number Collection Test-A performance time >30 s
- *2*) Hyperammonemia (venous ammonia concentration >50 mmol/L)
- *3*) Cooperative, hospitalized adult patients with liver cirrhosis diagnosed by clinical, histologic, and ultrasonographic findings (reduced dimensions of the liver as well as splenomegaly) and esophageal varices at stages 2 and 3 observed by endoscopy

Exclusion criteria

The exclusion criteria were as follows:

- *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 neurologic disease and metabolic disorders, diabetes mellitus, unbalanced heart failure, and/or respiratory failure or end-stage renal disease
- *4*) Alcoholic-toxic cirrhosis because toxic brain damage may interfere with the assessment of HE
- *5*) Severe HE
- *6*) Administration of anti-HE medications, such as neomycin and branched-chain amino acids
- *7*) Any additional precipitating factors, such as high protein intake (additional high-protein meals), constipation, or intake of psychostimulants, sedatives, antidepressants, benzodiazepines, benzodiazepines-antagonists (flumazenil), neuromuscular blocking agents, and certain antibiotics
- *8*) Patients with fever, sepsis, or shock were also excluded to avoid variations caused by body temperature
- *9*) Illiteracy

The study protocol was received and approved by the Institutional Review Board of the Hospital following the guidelines of the 1975 Declaration of Helsinki (16). All patients

gave written informed consent before any study procedures were initiated.

Study design

This was a randomized, double-blind, placebo-controlled study. The study was performed between June 2002 and December 2007. Patients meeting the inclusion criteria were randomly assigned to either a 90-d treatment with ALC (group A) or placebo (group B). Randomization was based on a computer-generated list. All study subjects were subdivided into 2 groups on the basis of the initial grade of HE: mild (grade 1; HE1) or moderate (grade 2; HE2) according to the West-Haven criteria (17). Group A consisted of patients with initial HE1 (ALC group: $n = 31$; placebo group: $n = 30$ placebo); group B consisted of patients with initial HE2 (ALC group: $n = 30$ patients; placebo group: $n = 30$ patients). The effectiveness of therapy was compared and evaluated separately in the different subgroups.

Methods

Clinical and laboratory assessment and automated electroencephalogram (EEG) analysis were performed for all patients. The diagnosis of HE grade was based on the evaluation of consciousness, intellectual functions, behavior, and neuromuscular functions and was made when appropriate laboratory and diagnostic testing excluded other causes of mental status changes. The investigators were blinded to the patients' ammonia concentrations. Patients whose clinical course was not consistent with HE were excluded. Mental status was assessed

and graded on admission according to the West Haven criteria introduced by Conn (18).

Prerandomization phase

The subjects were required to document all caloric intake with the use of a diary, which was completed every 2 d. This prerandomization period was designed to nullify the effects of dietary changes on metabolic markers. During the initial 2-wk phase, subjects were instructed by a dietitian to follow an ad libitum diet as follows: 25–30% total fat, <7% saturated fat, ≤10% polyunsaturated fat, ≤20% monounsaturated fat, 50–60% of total energy as carbohydrate, ≈15% of total energy as protein, and <200 mg cholesterol/d (19). Patients were seen by a dietitian every month; at each visit the dietitian provided instructions on dietary intake recording procedures as part of a behavior-modification program, and the patients' resulting food diaries were later used for counseling. All patients in both groups were given the same 1600-calorie diet and prescribed exercise plan. The subjects underwent weekly visits throughout the treatment period to assess adherence to the study protocol, to measure blood pressure, and to record adverse events.

Randomization phase

Throughout the trial, ALC was supplied in vials with 2 g ALC taken orally twice a day. All drugs and placebos were identical in appearance, and neither the investigators nor the patients were informed of the selected agent until the end of the study phase. Dosing instructions were provided with each patient pack. All

trial medication was instructed to be taken as prescribed. Subjects were considered compliant if the number of returned vials was between 80% and 120% of the planned treatment regimen. For the duration of the trial, any concomitant drugs were administered at the lowest possible therapeutic dose and, as much as possible, were not changed. Concomitant medications throughout the study included diuretics and β-blockers (**Table 1**).

TABLE 1
Concurrent medications taken by the patients at enrollment[1]

	Group A: ALC (n = 61)	Group B: placebo (n = 60)
	n	n
β-Blockers	20	18
Insulin	4	4
Furosemide	18	16
Lactulose	10	13

[1] ALC, acetyl-L-carnitine. There were no significant differences between the 2 treatment groups.

Fatigue assessment

Severity of fatigue

Severity of fatigue was measured by the Fatigue Severity Scale (FSS). The FSS is a self-assessed 9-question scale ranging from 1 (no signs of fatigue) to 7 (most disabling fatigue). Here, the total score ranged from 9 to 63 and is directly related to the severity observed (20).

Nature of fatigue

Wessely's test and Powell's test were used to examine fatigue, both mental and physical. The Wessely and Powell score consists of 2 scales measuring physical fatigue [8 items scored from 0 (no

fatigue) to 2 (highest possible fatigue); total score range: 0–16] and mental fatigue (5 items; total score range: 0–10) (21).

Measures of physical activity

Physical activity was assessed by using the 7-d Physical Activity Recall questionnaire (7-d PAR) and a pedometer. On the 7-d PAR, the patients self-reported moderate, hard, and very hard periods of physical activity performed during the 7-d period. The total duration of physical activity classified as "at least moderate intensity" was computed and used for analysis. This self-administered questionnaire has been shown to provide valid and reliable estimates of habitual physical activity (22). A pedometer (Digiwalker SW-200; Yamax Corporation, Tokyo, Japan) was used to obtain an objective measure of ambulatory physical activity. The subjects were instructed to wear the pedometer daily for 1 wk before treatment and for 1 wk before their scheduled 3-mo follow-up assessment. They were provided a diary to record their daily steps. The data are presented as the average steps taken daily. Physical function was assessed by using both performance-based and self-reported measures. The 6-min walk test (6MWT) measures the distance walked in 6 min on level ground, with stops to rest as needed. The subjects were told that the purpose of this test was to determine the distance they could walk in 6 min. They were instructed to "walk at their own pace in order to cover as much ground as possible" (23). The Short Physical Performance Battery (SPPB) is a battery of tests that has been used to assess lower extremity function in the older population (24). This battery uses a scale from 0 (poor) to

12 (excellent) to summarize the performance of 3 tasks (a 4-m walk, standing balance, and rising from a chair). For the 4-m walk, the subject walks a distance of 4 m at their normal pace to determine gait speed, computed as the time to complete the 4-m walk. For the standing balance test, the subjects placed their feet in a side-by-side position, followed by a semitandem position (heel of one foot along the side of the big toe of the opposite foot) and a tandem position (heel of one foot directly in front of the other). The subjects were required to hold the side-by-side position for 10 s before advancing to the semitandem position and to hold the semitandem position for 10 s before advancing to the tandem position. For the chair rise test, the subject was seated in a chair that was 18 in (≈45.72 cm) tall, with their arms crossed, and how quickly they could stand 5 times from sitting in the chair was assessed (24).

Neurophysiologic assessment

The EEG was recorded by using standardized techniques. Five electrodes were attached to the skin at the positions T3, T4, O1, O2, and Cz according to the international "10-20 system." Electrode impedance was kept lower than 5KQ. After the usual handpass filters (0.53–35 Hz) were applied, 2 runs of 100 s each were recorded and compared for reproducibility. Patients were graded into different studies of HE according to their mean dominant frequency (MDF) and the relative powers of delta and theta activity (25). The EEG is the only test that classifies HE in 5 grades of severity (from normal to coma), just as the clinical grading: grade 0 (normal, regular alpha rhythm), grade 1 (irregular background activity, alpha and theta rhythm), grade 2

(continuous theta activity, occasionally delta activity), grade 3 (prevalence of theta activity, transient polyphasis complexes of spikes and slow waves), and grade 4 (continuous delta activity, abundant complexes of spikes and slow waves) (26).

Liver function assessment

The Child-Pugh score was determined to assess the severity of cirrhosis, including 3 biochemical variables (serum albumin, bilirubin, and prothrombin time) and 2 clinical characteristics (presence or absence of ascites and clinical HE). A patient had Child-Pugh score A cirrhosis if the score was ≤6 points, Child-Pugh B cirrhosis if the score was 7–9 points, and Child-Pugh C cirrhosis if the score was >9 points. Patients without signs of ascites were scored as 2 points for ascites (27). We also evaluated the presence and severity of the porto-systemic shunt by portal vein flow, presence and size of the esophageal varices, and splenic size.

Venous ammonia concentration

Ammonia was measured by enzymatic determination of glutamate dehydrogenase in a rapid and interference-free photometric determination (340 nm) of NH_4^+ in native blood plasma according to the Da Fonseca-Wollheim method (28). For reasons of safety, blood was immediately refrigerated and transported to the laboratory for immediate measurement of NH_4^+ (within 15 min of blood withdrawal).

Efficacy assessment

Throughout the randomization phase of the study, thrice weekly alimentary diary cards were used to collect efficacy data. The primary efficacy measures were changes in activity, motivation, and physical and mental fatigue severity. Measurements were made at the beginning and at the end of the study period. Data were collected in the morning, after an overnight fast. Activity, motivation, physical and mental fatigue, and the severity of fatigue were assessed before and after treatment.

Tolerability assessment

Laboratory assessments were monitored on days 0, 30, 60, and 90. These data included blood tests (hemoglobin, hematocrit, white blood cell count, and thrombocytes) and liver function tests [alanine aminotransferase (AST), aspartate aminotransferase (AST), γ-glutamyl-transpeptidase, cholinesterase activity, serum bilirubin concentrations, prothrombin time, and partial thromboplastin time]. Electrocardiogram and blood pressure were monitored with the use of standard techniques.

Statistical analysis

We calculated that a simple size of ≥25 patients in each arm would be required to detect a difference in improvement in HE,

that is the proportion of patients with HE at 2 mo, with a 5% type 1 error and 90% power for a 2-tailed log-rank test. Descriptive statistics were prepared from the study sample, and the results are expressed as means ± SDs. The statistical significance in contingency tables was evaluated by using chi-square and Fisher exact test. Student's *t* test was used for unpaired data, and one-factor analysis of variance and the Mann-Whitney rank-sum test were used for comparisons of continuous variables. The statistical analyses were performed by using appropriate tests for repeated measures and by controlling for multiple comparisons by correction with the Duncan procedure. Differences in tolerability were assessed with a chi-square test comparing the proportions permanently withdrawn from all study drugs or placebos. Statistical Analysis System software version 6.11 (SAS Institute, Cary, NC) was used for all analyses.

3. Results

Baseline values

The 2 groups were homogeneous for demographic characteristics, etiology, casting of disease, Child-Pugh grade, anamnestic, and diagnostic criteria (**Table 2**). Differences in the composition of the 2 groups with respect to precipitant factors might be minimized, because the patient population was well defined by inclusion and exclusion criteria. Serum NH_4^+ fasting concentrations were not significantly different before the

treatment. No statistically significant differences were observed between the 2 groups about prothrombin time, serum albumin, bilirubin, AST, and ALT. No statistically significant differences in the administered neuropsychologic test or in the EEG were observed between the 2 groups.

TABLE 2
Baseline characteristics of the patients[1]

Characteristic	Group A: ALC (n = 61)	Group B: placebo (n = 60)
Sex (male/female)	32/29	33/27
Age (y)	40–66	41–67
SBP (mm Hg)	140 ± 16[2]	136 ± 18
DBP (mm Hg)	80 ± 7	77 ± 9
HR (beats/min)	87 ± 16	84 ± 15
NCT-A (s)	48 ± 12	47 ± 14
Cirrhosis etiology (n)		
Posthepatitis B	12	10
Posthepatitis C	30	35
Alcoholism	5	4
Cryptogenetic	14	11
Child-Pugh class		
A	20	21
B	36	28
C	5	11

[1] ALC, acetyl-L-carnitine; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; NCT-A, number collection test-A. There were no significant differences between the 2 treatment groups.
[2] Mean± SD (all such values).

Neurophysiologic response

In the comparison between group A (treated with ALC) and group B (treated with placebo) we observed in HE1 an improvement in EEG grading in 45% of patients in the ALC group and in 13% of patients in the placebo group [odds ratio (OR): 5.35; 95% CI: 1.50, 19], whereas in HE2 there was an improvement in EEG grading in 66% of patients in the ALC group compared with 27% of patients in the placebo group (OR: 5.5; 95% CI: 1.81, 1.37) (**Table 3**).

TABLE 3
Electroencephalogram, fatigue, and physical results in the patient subgroups[1]

Medication	Improved	Not improved	Total subjects	Improved:not improved	Odds ratio
	n (%)	*n*	*n*		
Electroencephalogram results in the patient subgroups					
Initial HE grade 2					
Group A: ALC	20 (66)	10	30	2	5.5
Group B: placebo	8 (27)	22	30	0.36	
Total	28	32	60		
Initial HE grade 1					
Group A: ALC	14 (45)	17	31	0.82	5.35
Group B: placebo	4 (13)	26	30	0.15	
Total	18	43	61		
Fatigue results in the patient subgroups					
Initial HE grade 2					
Group A: ALC	25 (83)	5	30	5	10
Group B: placebo	10 (33)	20	30	0.5	
Total	35	25	60		
Initial HE grade 1					
Group A: ALC	20 (64)	11	31	1.81	7.27
Group B: placebo	6 (20)	24	30	0.25	
Total	26	35	61		
Physical activity results in the patient subgroups					
Initial HE grade 2					
Group A: ALC	23 (77)	7	30	3.28	7.66
Group B: placebo	9 (30)	21	30	0.42	
Total	32	28	60		
Initial HE grade 1					
Group A: ALC	22 (71)	9	31	2.44	9.77
Group B: placebo	6 (20)	24	30	0.25	
Total	28	33	61		

[1] ALC, acetyl-L-carnitine; HE, hepatic encephalopathy.

L-Carnitine in plasma and urine

In the ALC group, significant differences were observed in the following markers after treatment compared with baseline in both HE1 and HE2: free plasma L-carnitine ($P < 0.001$), plasma concentrations of total plasma L-carnitine ($P < 0.001$), plasma long-chain acylcarnitine (LCAC) ($P < 0.001$), and short-chain acylcarnitine (SCAC) ($P < 0.05$). Only in HE2 did we observe significant differences in free urinary L-carnitine ($P < 0.001$). In the placebo group (in both HE1 and HE2), the plasma concentrations of free L-carnitine and LCAC and the urinary

excretion of free L-carnitine and SCAC were not significantly different from baseline. At the end of the study period, compared with placebo, the ALC-treated patients showed significant improvements in the following markers in HE1 and HE2: free plasmaL-carnitine (3.8 compared with 0.7 μmol/L in HE1 and 5.4 compared with 0.8 μmol/L in HE2; $P < 0.001$), plasma concentrations of total L-carnitine (4.4 compared with 0.9 μmol/L in HE1 and 6.2 compared with 1.1 μmol/L in HE2; $P < 0.001$), and plasma SCAC (0.4 compared with 0.1 μmol/L in HE1 and 0.5 compared with 0.2 μmol/L in HE2; $P < 0.001$) (**Table 4**).

TABLE 4
Comparison of plasma and urinary concentrations of L-carnitine between treatment groups[1]

	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and 30 HE2)			
Variable	Before treatment	After 90 d of treatment	Before treatment	After 90 d of treatment	P for time[2]	P for group × time[2]
Free plasma L-carnitine (μmol/L)						

	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and 30 HE2)			
Variable	Before treatment	After 90 d of treatment	Before treatment	After 90 d of treatment	P for time[2]	P for group × time[2]
HE1	38.5 ± 3.9	42.3 ± 2.6[3]	38.3 ± 3.8	39 ± 3.5[4]	<0.001	<0.001
HE2	30.8 ± 4.3	36.2 ± 2.8[3]	31.1 ± 5	31.9 ± 4.2[4]	<0.001	<0.001
Plasma SCAC (μmol/L)						
HE1	7.6 ± 0.5	8 ± 0.5[3]	7.2 ± 0.6	7.3 ± 0.6[4]	<0.05	<0.001
HE2	6.5 ± 0.7	7 ± 0.5[3]	6.2 ± 0.5	6.4 ± 0.6[4]	<0.05	<0.001
Plasma LCAC (μmol/L)						
HE1	1.8 ± 0.3	2.1 ± 0.2[3]	2 ± 0.3	2.1 ± 0.4	<0.001	1.000
HE2	1.7 ± 0.4	2 ± 0.3[3]	1.8 ± 0.3	1.9 ± 0.3	<0.001	0.202
Total plasma L-carnitine (μmol/L)						
HE1	48.1 ± 4	52.5 ± 2.8[3]	47.6 ± 4.3	48.5 ± 3.6[4]	<0.001	<0.001
HE2	39.1 ± 4.5	45.3 ± 2.7[3]	39.1 ± 5.0	40.2 ± 4.2[4]	<0.001	<0.001
Free urinary L-carnitine (μmol/L)						
HE1	11.3 ±	11.6 ±	11.3 ±	11.4 ±	0.054	0.235

	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and 30 HE2)			
Variable	Before treatment	After 90 d of treatment	Before treatment	After 90 d of treatment	P for time[2]	P for group × time[2]
	0.6	0.6	0.9	0.7		
HE2	10.9 ± 0.5	11.3 ± 0.4[3]	11.1 ± 0.6	11.3 ± 0.5	<0.001	1.000
Urinary SCAC (μmol/L)						
HE1	10.7 ± 0.5	10.8 ± 0.2	10.8 ± 0.5	11.1 ± 0.4[4]	0.305	<0.001
HE2	11 ± 0.4	11.2 ± 0.4	11.1 ± 0.5	11.4 ± 0.5	0.058	0.092

- ↵1 All values are means ± SDs. ALC, acetyl-L-carnitine; HE1, patients with mild hepatic encephalopathy; HE2, patients with moderate hepatic encephalopathy; SCAC, short-chain acylcarnitine; LCAC, long-chain acylcarnitine. There were no significant differences between groups at baseline.
- ↵2 Determined with ANOVA.
- ↵3 Significantly different from before treatment, $P < 0.05$.
- ↵4 Significantly different from ALC treatment, $P < 0.05$.

Effects of ALC on fatigue

At the end of treatment in the group treated with ALC in HE1, we observed significant differences from baseline in the physical fatigue score ($P < 0.001$), mental fatigue score ($P < 0.001$), and fatigue severity scale ($P < 0.001$); in HE2, we observed significant differences in the physical fatigue score ($P < 0.05$), mental fatigue score ($P < 0.001$), and fatigue severity scale ($P < 0.001$). After 90 d, significant differences were observed between the ALC-treated patients in HE1 and the placebo-treated

patients in mental fatigue score (−1.7 compared with −0.3; $P < 0.05$) and fatigue severity scale (−6.4 compared with 2.3; $P < 0.001$), whereas in HE2 significant differences were observed in the fatigue severity scale (−8.1 compared with −5.1; $P < 0.001$) (**Table 5**). In the comparison between group A (treated with ALC) and group B (treated with placebo), we observed in HE1 an improvement in fatigue severity in 64% of patients in the ALC group compared with 20% of patients in the placebo group (OR: 7.27; 95% CI: 2.44, 23.16), whereas in HE2 there was an improvement in fatigue severity in 83% of patients in the ALC group compared with 33% of patients in the placebo group (OR: 10; 95% CI: 2.94, 34) (Table 3).

TABLE 5
Comparison of evaluated parameters within groups and between groups[1]

	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and HE2)			
	Before treatment	90 d after treatment	Before treatment	90 d after treatment	P for time[2]	P for group × time[2]
Physical fatigue score (0–16)						
HE1	11.8 ± 1.8	9.5 ± 2.2[3]	9.9 ± 2.4	9.3 ± 1.4	<0.001	0.675
HE2	10.6 ± 2.3	8.7 ± 1.1[3]	10.4 ± 2.5	8.9 ± 1.7	<0.001	0.591
Mental fatigue score (0–10)						

	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and HE2)			
	Before treatment	90 d after treatment	Before treatment	90 d after treatment	P for time[2]	P for group × time[2]
HE1	7.3 ± 1.5	5.6 ± 1.2[3]	6.1 ± 1.3	6.4 ± 1.5[4]	<0.001	<0.05
HE2	7.1 ± 1.5	6.2 ± 1.1[3]	7.8 ± 1.2	5.8 ± 1.3	<0.05	0.203
Fatigue severity scale (9–63)						
HE1	40.8 ± 4	34.4 ± 2.9[3]	41.8 ± 4.9	44.1 ± 4.5[4]	<0.001	<0.001
HE2	53.6 ± 5	45.5 ± 4.4[3]	54.5 ± 4.9	49.4 ± 4.8[4]	<0.001	<0.001
7D/PAR						
HE1	214.8 ± 24.4	231.9 ± 21.3[3]	205.6 ± 23.6	203.1 ± 15.2[4]	<0.05	<0.001
HE2	166.8 ± 22.4	197.4 ± 18.4[3]	196.6 ± 29.6	205.6 ± 29.1	<0.001	0.197
Pedometer (average daily steps)						
HE1	4902.5 ± 481.5	4996.7 ± 492.9	5006.3 ± 501.6	5020 ± 477.3	0.450	0.852
HE2	3846.5 ± 460	4199.6 ± 364.6[3]	4170.6 ± 449.7	4178 ± 333.1	<0.001	0.812
6MWT						
HE1	372.5 ± 21.4	382 ± 21.6	373 ± 18.8	377 ± 15.4	0.087	0.304
HE2	286 ± 46.7	305.9 ± 34.8	276.9 ± 45.7	279.2 ± 37.2[4]	0.063	<0.05

	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and HE2)			
	Before treatment	90 d after treatment	Before treatment	90 d after treatment	P for time[2]	P for group × time[2]
SPPB						
HE1	7 ± 1	9.1 ± 1.5[3]	7 ± 1.3	7.2 ± 1.1[4]	<0.001	<0.001
HE2	6.3 ± 1.9	8 ± 1.7[3]	7.5 ± 1.7	8 ± 1.5	<0.001	1.000

- ↵1 All values are means ± SDs. ALC, acetyl-L-carnitine; HE1, patients with mild hepatic encephalopathy; HE2, patients with moderate hepatic encephalopathy; 7D/PAR, 7-d Physical Activity Recall questionnaire score; 6MWT, 6-min walk test; SPPB, Short Physical Performance Battery. There were no significant differences between groups at baseline.
- ↵2 Determined with ANOVA.
- ↵3 Significantly different from before treatment, $P < 0.05$.
- ↵4 Significantly different from ALC treatment, $P < 0.05$.

Effects of ALC on physical activity

At the end of treatment in the group treated with ALC in HE1 we observed significant differences in 7D/PAR ($P < 0.05$) and SPPB ($P < 0.001$); in HE2 the significant differences were in 7D/PAR ($P < 0.001$), pedometer ($P < 0.001$), and SPPB ($P < 0.001$). After 90 d, significant differences between the ALC-treated patients in HE1 and the placebo-treated patients were observed in 7D/PAR (17.1 compared with −2.5; $P < 0.001$) and SPPB (2.1 compared with 0.2; $P < 0.001$); in HE2 the significant differences were in 6MWT (19.9 compared with 2.3; $P < 0.05$) (Table 5). In the comparison between group A (treated with ALC) and group B (treated with placebo), we observed in HE1 an improvement in physical activity in 71% of patients in the ALC group compared

with 20% of patients in placebo group (OR: 9.77; 95% CI: 2.99, 31.94), whereas in HE2 there was an improvement in physical activity in 77% of patients in the ALC group compared with 30% of patients in the placebo group (OR: 7.66; 95% CI: 2.42, 24.24) (Table 3).

Biochemical response

Effects of ALC on ammonia

In HE1 and HE2 at the end of treatment with ALC, we observed a significant decrease in NH_4^+ ($P < 0.001$). Moreover, in the comparison between group A (treated with ALC) and group B (treated with placebo), significant differences in NH_4^+ were observed in HE1 (−23.4 compared with −3.5; $P < 0.001$) and in HE2 (−28.8 compared with −5.7; $P < 0.001$) (**Table 6**).

TABLE 6
Comparison of laboratory values within and between groups[1]

	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and 30 HE2)			
	Before treatment	90 d after treatment	Before treatment	90 d after treatment	P for time[2]	P for group × time[2]
NH_4^+(mg/dL)						
HE1	78.3 ± 10.9	54.9 ± 10.1[3]	71.4 ± 9.8	67.9 ± 10.5[4]	<0.001	<0.001
HE2	111.2 ± 14.8	82.4 ± 18.3[3]	99.4 ± 12.9	93.7 ± 11.6[4]	<0.001	<0.001
Albumin (g/dL)						

	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and 30 HE2)			
	Before treatment	90 d after treatment	Before treatment	90 d after treatment	P for time[2]	P for group × time[2]
HE1	3.5 ± 0.3	3.7 ± 0.4[3]	3.5 ± 0.3	3.4 ± 0.3[4]	<0.05	<0.05
HE2	3.5 ± 0.3	3.6 ± 0.2	3.7 ± 0.2	3.7 ± 0.2	0.134	0.058
Prothrombin time (%)						
HE1	74.1 ± 6.8	74.5 ± 5.3	61.6 ± 5.7	62.7 ± 4.8[4]	0.797	<0.001
HE2	65 ± 5.2	65.4 ± 3.9	59.3 ± 5	61.1 ± 4.6[4]	0.735	<0.001
Bilirubin (mg/dL)						
HE1	2.1 ± 0.5	2 ± 0.4	1.7 ± 0.3	1.7 ± 0.2[4]	0.388	<0.001
HE2	2.2 ± 0.6	2 ± 0.5	2.2 ± 0.5	2.3 ± 0.4[4]	0.166	<0.05
AST (IU/L)						
HE1	98.6 ± 12.8	89.4 ± 8.7[3]	105.3 ± 12.4	100.7 ± 13.1[4]	<0.05	<0.001
HE2	124.4 ± 22.4	114.8 ± 17.1	154.9 ± 10.6	147 ± 9.6[4]	0.067	<0.001
ALT (IU/L)						
HE1	111.5 ± 10.7	99.4 ± 7.3[3]	105.2 ± 10.6	92.6 ± 19.5	<0.001	0.075
HE2	140.7 ± 13.8	125.5 ± 7.5[3]	136.8 ± 23.5	130.6 ± 17.2[4]	<0.001	<0.05

- ↵1 All values are means ± SDs. ALC, acetyl-L-carnitine; HE1, patients with mild hepatic encephalopathy; HE2, patients with moderate hepatic encephalopathy; AST, aspartate transaminase; ALT, alanine transaminase. There were no significant differences between groups at baseline.
- ↵2 Determined with ANOVA.

- ↵3 Significantly different from before treatment, $P < 0.05$.
- ↵4 Significantly different from ALC treatment, $P < 0.05$.

Tolerability

Three patients in the ALC group (1 with mild HE and 2 with moderate HE) withdrew from the study because of abdominal pain. One patient in the placebo group withdrew from the study because of headaches. In the placebo group, we observed occasional abdominal pain, cramping, diarrhea, and flatulence. At follow-up 1 mo after treatment ended, 2 patients in the ALC group and 5 patients in the placebo group experienced moderate HE.

4. Discussion

Fatigue is a multidimensional syndrome and can be described in terms of perceived energy, mental capacity, psychological status, sport, and physical exercise. The suggestion that ammonia accumulation has a significant role in fatigue is not new. It was established that there was an intensity-dependent relation between plasma ammonia concentration and exercise (29). In our study we observed reductions in severity in both physical and mental fatigue and improvements in physical activity and physical function after ALC administration. We also observed an improvement in 7D/PAR, in average daily steps measured by pedometer and in SPPB, whereas poor improvements were recorded in the placebo recipients. Fatigue is a subjective

sensation with decreased energy, decreased concentrations, and decreased motivation; it can impair daily functioning and lead to negative effects on quality of life and self-care capabilities (30). Numerous mechanisms and contributory factors have been implicated in fatigue, including *1*) build-up of peripheral toxins and metabolic byproducts and changes in peripheral environment (31, 32), *2*) centrally mediated self regulation (33), *3*) inflammatory cytokine production (34–36), *4*) alterations in neurotransmitter metabolism (37), and *5*) periphery-regulated central drive control (38). The suggested mechanisms include an imbalance in energy metabolism due to increased energy requirements, decreased availability of metabolic substrates, and an abnormal production of substances that impair metabolic homeostasis or normal muscle functioning. The ALC treatment in our study significantly reduced both physical and mental fatigue. L-Carnitine and ALC are often used to foster exercise performance.

There is evidence of a beneficial effect of L-carnitine and ALC supplementation in training competition and recovery from strenuous exercise and in regenerative athletics (39). A great deal of research has investigated the effects of L-carnitine and ALC supplementation on exercise performance—the main premise being that increasing L-carnitine availability would increase fat oxidation during prolonged exercise, spare glycogen stores, and thus delay the onset of fatigue (40). The increase in ALC formation during high-intensity exercise, which occurs to a greater extent in type 1 muscle fibers (41), is directly related to an increase in muscle acetyl-CoA (42, 43), which suggests that

the rate of acetyl-CoA formation from pyruvate oxidation, catalyzed by the pyruvate dehydrogenase complex, is in excess of its utilization by the tricarboxylic acid cycle. In our study we observed a significant decrease in serum ammonia concentrations and a significant improvement in mental function in patients treated with ALC. Ammonia is a product of the metabolism of nitrogen-containing compounds and is involved in many metabolic reactions. However, ammonia is toxic at elevated concentrations and must be removed from the body (44–46). In patients with HE, brain and muscle cells are involved in the metabolism of ammonia to a greater extent than normal. These "ammonia sinks" use the amino acid glutamate to detoxify ammonia by converting it to glutamate (47). Skeletal muscle metabolizes ammonia in patients with cirrhosis. Loss of lean body mass depletes this ammonia sink and increases the ammonia load to the brain, thereby worsening HE. HE is the result of multiple biochemical influences on central neurotransmitter systems.

In addition to the neurotoxic effects of ammonia, derangements in the γ-aminobutyric acid–ergic (GABA-ergic), serotoninergic, and dopaminergic systems are evident. Reduced detoxification of neurotoxic substances, particularly ammonia, in the cirrhotic liver and subsequently alterations in several neurotransmitter systems and brain edema are supposed to be major factors in the development of HE (48–50). Neurotransmitter systems are affected by increased intracerebral concentrations of ammonia, including the GABA-ergic, glutamatergic, and serotoninergic systems (51–53). Some studies estimated that ≈50% of ammonia

may be metabolized in muscle to form glutamine via the glutamine synthetase reaction (54). In animal models of acute and chronic liver failure, hyperammonemia is associated with a rapid increase in glutamine synthetase activity in the skeletal muscle, which results in an increase in the muscle's capacity to remove ammonia (55). In patients with cirrhosis, skeletal muscles may metabolize more ammonia than the cirrhotic liver (56). Previous studies showed that ALC decreases the severity of physical and mental fatigue (57–59). ALC mobilizes acetyl groups and stimulates phospholipid synthesis and increases acetyl-coenzyme A and choline uptake and acetylcholine release (60). It is also involved in the synthesis of glutamate; in fact, the acetyl moiety of ALC is metabolized mainly to glutamate, but also to glutamine, aspartate, and GABA via the tricarboxylic acid cycle. Studies of the role of ALC in aged rat brains showed, in the brain regions with lower amino acid concentrations, that the release of neurotransmitter amino acids is below normal and ALC produces an increase in the extracellular concentration of neurotransmitter glutamate. On the other hand, ALC decreases glutamate dehydrogenase activity in the intrasynaptic mitochondria of the rat brain, which suggests that ALC interferes with glutamate metabolism. The increase in glutamate, caused by elevated plasma ALC concentrations, results in protection against excitotoxic cell death. This is possible through the direct antagonism of glutamate receptors and the activation of GABA receptors that cause neuronal hyperpolarization and therefore resistance to NMDA receptor activation or to inhibition of secondary events. These secondary events could include

activation of the mitochondrial permeability transition that can cause the release of mitochondrial cytochrome *c* and stimulation of reactive oxygen species production. These studies showed that the administration of 2 g ALC twice a day attenuated the effect of hyperammonemia.

The major finding and new discovery of the present study was that ALC supplementation can beneficially affect the severity of both physical and mental fatigue. These findings might have been influenced by the limitations in the methods used to assess physical activity. The pedometer might have inaccurately measured the magnitude of physical activity before and after treatment. For example, the pedometer might not have accurately assessed activities other than level walking, the positioning of the pedometer could have affected the accuracy of the measurement, and subjects were required to accurately self-report their daily steps to the investigators (61). It has also been shown that cirrhosis individuals inaccurately report their physical activity, which could have contributed to the patterns observed in this study (62). Therefore, the use of objective monitoring of physical activity should be considered for future studies. With regard to the whole study population, a clear treatment effect in favor of ALC was shown regarding the improvement in EEG grading, fatigue severity, and physical activity. Accordingly, a superiority of ALC in comparison with placebo was shown in the subgroups with HE2. Otherwise, it could be shown that the response to ALC in patients with HE1 is smaller than that in those patients with HE2. On the basis of the ORs, it was observed that the

greater the initial mental state gradation of HE, the greater the effect of ALC on EEG grading and fatigue severity.

In conclusion, the administration of ALC in compensated patients with cirrhosis could enhance the tolerance to protein load and low ammonia concentrations and improve neurologic symptoms in patients with HE and was at least as useful as placebo in the long-term treatment of both chronic grade 1 and grade 2 HE. The patients with HE treated with ALC showed a decrease in the severity of both mental and physical fatigue, an increase in physical activity, and an improvement in daily functioning. Treatment with ALC may lead to a positive spiral: an improvement in physical activity that leads to a reduction in the severity of fatigue, which leads to further activity (63). The role of ammonia in the neuromuscular activity of patients with HE remains to be determined in future studies.

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CHAPTER II

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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1. 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 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.

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

Table 1

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

2. 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, cholinesterase 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).

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

Parameters	Group A ALC	Group B placebo
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

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

	ALC Group A		Placebo Group B	
Grade	**Before treatment**	**After treatment**	**Before treatment**	**After treatment**
4	2	0	2	1

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]

	Group A ALC		Group B Placebo	
	Before treatment	**After treatment**	**Before treatment**	**After treatment**
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

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, $P < 0.001$) 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).

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]

	Group A ALC		Group B Placebo	
	Before treatment	**After treatment**	**Before treatment**	**After treatment**
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

Discussion

We observed a significant improvement in neuropsychological response in patients with severe HE treated with 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-, hypo-tonicity) 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.

Conflicts of interest

The authors disclose no conflicts.

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CHAPTER III

Acetyl-L-carnitine in hepatic encephalopathy*

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Abstract

Hepatic encephalopathy is a common complication of hepatic cirrhosis. The clinical diagnosis is based on two concurrent types of symptoms: impaired mental status and impaired neuromotor function. Impaired mental status is characterized by deterioration in mental status with psychomotor dysfunction, impaired memory, and increased reaction time, sensory abnormalities, poor concentration, disorientation and coma. Impaired neuromotor function include hyperreflexia, rigidity, myoclonus and asterixis. The pathogenesis of hepatic encephalopathy has not been clearly defined. The general consensus is that elevated levels of ammonia and an inflammatory response work in

synergy to cause astrocyte to swell and fluid to accumulate in the brain which is thought to explain the symptoms of hepatic encephalopathy. Acetyl-L-carnitine, the short-chain ester of carnitine is endogenously produced within mitochondria and peroxisomes and is involved in the transport of acetyl-moieties across the membranes of these organelles. Acetyl-L-carnitine administration has shown the recovery of neuropsychological activities related to attention/concentration, visual scanning and tracking, psychomotor speed and mental flexibility, language short-term memory, attention, and computing ability. In fact, Acetyl-L-carnitine induces ureagenesis leading to decreased blood and brain ammonia levels. Acetyl-L-carnitine treatment decreases the severity of mental and physical fatigue, depression cognitive impairment and improves health-related quality of life. **The aim of this review was to provide an explanation on the possible toxic effects of ammonia in HE and evaluate the potential clinical benefits of ALC**.

Keywords

L-carnitine Acetyl-L-carnitine Ammonia Hepatic encephalopathy Cirrhosis

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1. Introduction

Hepatic encephalopathy (HE) is a debilitating complication of cirrhosis which presents as a spectrum of neurological and neuropsychiatric dysfunction, affecting the patients consciousness, intellect, personality and neuromuscular activity. The therapeutic armamentarium against hepatic encephalopathy is limited; however, the new knowledge concerning the pathogenesis of HE, its clinical heterogeneity, and variable assessment of its severity, have opened new horizons in the manner and in the type of treatment.

Many details of the pathophysiology leading to encephalopathy remain unclear. Some factors can contribute to pathogenesis of HE: ammonia, glutamine, manganese, false neurotransmitters, inflammation, short chain fatty acids, oxidative stress, mercaptanes, neurosteroids, or low grade edema (McPhail et al. *2010*; Norenberg et al. *2004*). In the kaleidoscope of most of the pathogenic mechanisms hyperammonemia plays a central role (Fig. 1). In order to counteract the protean characteristics of ammonia, our group has used the L-carnitine and its derivatives in HE. L-carnitine is a versatile endogenous molecule present in mammalian metabolism. Carnitine, a branched non essential amino acid, is synthesized from the essential amino acids lysine and methionine in kidney, liver, and brain (Rebouche *1992*). L-Carnitine (LC), acylcarnitines, and various carnitine enzymes

constitute the carnitine system that play a pivotal role in cellular energy production. The system is ubiquitous and the mitochondrial carnitine system has an obligatory role in beta oxidation of long-chain fatty acids by their transport into the mitochondrial matrix. LC and its esters are present in different concentrations in human serum (L-carnitine/acetyl-L-carnitine / propionil-L-carnitine = 5:1:0,1). Carnitines are involved in the removal of accumulated toxic fatty acyl-CoA metabolites and helping in the balance between free and acyl-CoA. The toxic effects of poorly metabolized acetyl groups can be lowered with transesterification from CoA and excretion of ALC esters by carnitine acetyltransferase (CAT) and carnitine palmitoyltransferases (CPT-1 and CPT-2). **The aim of this review was to provide an explanation on the possible toxic effects of ammonia in HE and evaluate the potential clinical benefits of ALC** (Fig. 2).

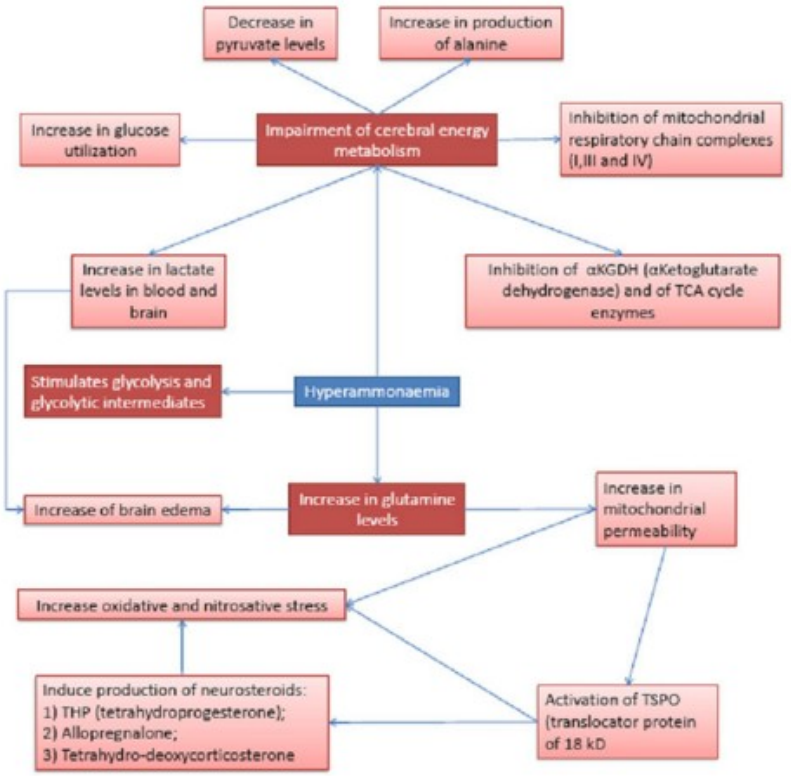

Fig. 1
Role of hyperammonaemia in the genesis of hepatic encephalopathy

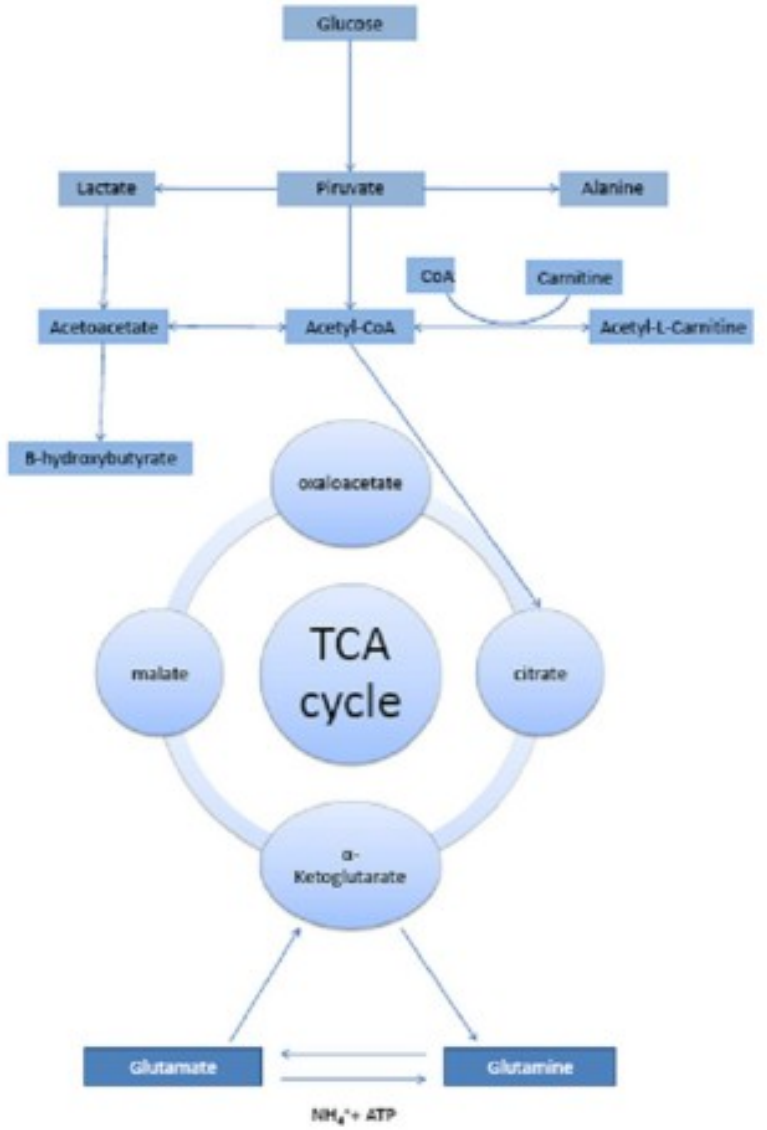

Fig. 2
Role of Acetyl-L-carnitine in the glucose metabolism and in the tricarboxylic acid cycle (TCA)

Toxic effects of hyperammonemia

Many mechanism have been proposed to explain the toxic effect of hyperammonaemia on brain development and function (Jones and Mullen *2012*). Such mechanisms include direct toxic effects of the ammonium ion on:

1)

The inhibitory and excitatory neurotransmission effects on the glutamate neurotransmitter system, on the cholinergic system, on the GABA-A receptor.

2)

The interference with cerebral metabolism through both inhibition of α-ketoglutarate dehydrogenase and inhibition of malate-aspartate shuttle

3)

The adverse effects on astrocytic function due to inhibition of astrocytic glutamate uptake, to increased expression of "peripheral-type" benzodiazepine receptors.

The mammalian brain remove excess blood-borne ammonia by glutamine formation (Rama Rao et al. *2012*). The increase in the level of ammonia and glutamate in the brain can reduce the activity of cytochrome C oxidase (COX) and the expression of its mRNA thus violating the energy supply to cerebral structures. The change in the activity of the respiratory chain enzymes is

more pronounced in the neuronal mitochondria rather than in the synaptosomal ones.

The first reports implicating a role for carnitine in th eregulation of serum ammonia followed observations of a Reye's syndrome, of a Reye-like syndrome due to carnitine deficiency. Similar observations have been made in patients receiving valproic acid (Matsuda and Ohtani *1986*). A similar presentation has been described in subjects with deficits in enzymes involved in mitochondrial carnitine transport. Disruption of the carnitine transport system results in the cytosolic accumulation of unoxidized fatty acyl-CoA molecules. These metabolites are believed to inhibit the urea cycle, thereby impairing an important mechanism of ammonia excretion (Limketkai and Zucker *2008*)

Rationale of acetyl-L-carnitine in the hepatic encephalopathy treatment

Acetyl-L-carnitine (ALC), the short-chain ester of carnitine is endogenously produced within mitochondria and peroxisomes and is involved in the transport of acetyl -moieties across the membranes of these organelles. ALC represents an acetylated form of LC. ALC is the most important carnitine ester found in the tissues of animals and A LC is able to cross the blood–brain barrier and reach the cerebral regions, where the acetylic group may be used. ALC facilitates the uptake of Acetyl-CoA into the mitochondria during fatty acid oxidation, enhances acetylcholine production, and stimulates protein and membrane phospholipids synthesis, provides a substrate reservoir for cellular energy

production, thereby preventing excessive neuronal cell death (Di Cesare Mannelli et al. *2010*; Fiskum et al. *2004*). Treatment with ALC improved neurological outcome and energy metabolism in various animal models. In the portacaval shunted rat with encephalopathy L-carnitine prevents high ammonia levels and normalizes alanine and lactate levels (Therrien et al. *1997*). In rat brain cells, the acetyl moiety of ALC may be used for biosynthesis of acetylcholine, fatty acids and amino acids (Scafidi et al. *2010*). ALC administration altered rat brain energy homeostasis by increasing phosphocreatine and decreasing lactate and inorganic phosphate levels and stimulating glycogen synthesis (Aureli et al. *1998*). In mice Carnitine and choline derivatives containing a trimethylamine group prevent acute ammonia toxicity (Miñana et al. *1996*). Moreover the protective effect of some of these compounds is attained at low doses, making it unlikely that the protection may be due to an osmotic effect (Llansola et al. *2002*). In the sparse-fur mutant mice with ornitine transcarbamylase L-carnitine treatment corrects defects in energy metabolites and hyperammonemia (Ratnakumari et al. *1993*) and reverses suppression of urea cycle enzyme expression. In rodents Lcarnitine supplementation appeared to prevent ammonia toxicity on three levels:**Activation of cycle enzymes** (Ratnakumari et al. *1993*); **Interaction with glutamate receptors** (Rodrigo et al. *2009*); **Reduction of free radicals** (Rose and Felipo *2005*). Six randomized controlled studies have been performed to evaluate the ALC administration in HE (Table 1). All patients received **4 g/daily of ALC**:**two studies focused on a population with minimal**

HE (Malaguarnera et al. *2008*, *2011a*), **one study focused on subjects with mild and moderate HE** (Malaguarnera et al. *2011b*), **one study on patients with severe HE** (Malaguarnera et al. *2011c*). **Improvement in quality of life**, **anxiety and depression**(Malaguarnera et al. *2011a*) **and cognitive functions** (Malaguarnera et al. *2008*) **has been observed In patients with HE treated with ALC**. **Subjects with mild and moderate HE treated with ALC showed significant improvement in physical and mental fatigue**. **Subjects with severe HE**, **after ALC administration**, **showed significant improvements in EEG**, **cognitive and memory functions**, **in visual scanning and tracking and in computing ability**. These studies demonstrated that ALC administration at supraphysiological concentration reduce serum ammonia levels and show the protective effect against ammonium toxicity and consequently against glutamate neurotoxicity. The excess of extracellular glutamate under ammonium ion exposure is excitotoxic through activation of N-Methyl-D-Aspartate receptors and leads to alteration in nitric oxide metabolism, disturbances in Na+/K+ ATPase, ATP shortage, mitochondrial disfunctions, free radical accumulation and oxidative stress (Rose and Felipo *2005*). Furthermore, ammonia exposure of the brain tissue can lead to alteration of other glutamate receptors like AMPA and mGluR. The glutamatergic excitotoxicity under ammonia exposure can also alter other neurotransmission systems like the activation of GABA or benzodiazepine receptors (Rodrigo et al. *2009*; Cauli et al. *2009*). ALC may have a dual protective effect by enhancing

the energy dynamics of the cell and also inhibiting cell membrane hyperexcitability. Excitotoxic damage via upregulation of glutamate/N-methyl-D-aspartate (NMDA) receptors is heavily dependent on the energy state of the cell. In fact, ALC induces ureagenesis leading to decreased blood and brain ammonia levels (Table 1) (Malaguarnera et al. *2006*, *2008*, *2009*, *2011a*, *b*, *c*).

Table 1

Randomized clinical trials on the effects of ALC in patients with HE

Study – Journal – Year	HE grading	Nº of patients	Dose	Duration	Route of administration	Biohumoral effects	Neurophysiological effect	Neuropsyciatric effects
Malaguarnera - Digestive disease science (2006)	Coma	24 - ALC (*n* = 13) vs. placebo (*n* = 11)	4 g daily	3 days	Intravenous	Decrease in serum ammonia and serum urea	Improvement of EEG grade in the group treated with LAC	Decrease in Glasgow score
Malaguarnera Digestive disease science (2008)	Minimal HE	125 – ALC (*n* = 65) vs. placebo (*n* = 60)	2 g twice daily	90 days	Oral	Significant decrease in: bilirubin serum levels; AST; NH(4) serum levels. Increase in: albumin serum levels	No significant differences were observed in EEG of both patients treated with ALC or with placebo.	Reduction in: TMT-A; TMT-B. Increase in: MMSE test; in SDM Test, in BDT; in AVLLT and in AVL T.

Study – Journal – Year	HE grading	Nº of patients	Dose	Duration	Route of administration	Biohumoral effects	Neurophysiological effect	Neuropsychiatric effects
Malaguarnera Eur J Gastroenterol Hepatol. (2009)	Coma	48-ALC+BCAA (*n* = 24) versus BCAA (*n* = 24)	4 g in 5% glucose (500 ml) + BCAA in water (500 ml)	1 day	Intravenous	significant decrease of ammonia serum levels	significant improvement of EEG in the group treated with LAC	Increase in Glasgrow's score
Malaguarnera Am J clinnutr (2011b)	mild and moderate	121-HE1 (31) HE2 (30) HE2 received ALC versus HE1	2 g twice daily	90 days	Oral	significant decrease in NH4+ and bilirubin; in HE1 decrease in	Improvement in EEG grading in groups treated with LAC	Improvement in physical and mental fatigue

Study – Journal – Year	HE grading	Nº of patients	Dose	Duration	Route of administration	Biohumoral effects	Neurophysiological effect	Neuropsychiatric effects
		(30) and HE2 (30) with placebo				AST and ALT, in HE2 decrease in ALT		
Malaguarnera Scand J gastr (2011a)	Minimal HE	67 – ALC (*n* = 33) or placebo (*n* =34)	2 g two ice daily	90 days	Oral	Significant decrease of: ammonia serum levels, urea, AST	No statistical differences have been observed in the two groups in EEG	Improvement of physical function ($p < 0.001$); role physical; general health; social function; role emotional; mental health;
Malaguarner	Severe H	60 - ALC	2 g tw	90 days	Oral	Significant	significant improvem	Improvement in:

Study – Journal – Year	H E grading	Nº of patients	Dose	Duration	Route of administration	Biohumoral effects	Neurophysiological effect	Neuropsychiatric effects
a Metabbrain disease (2011c)	E	twice a day (*n* = 30) or placebo (*n* = 30)	ice daily			decrease of: ammonia serum levels, AST, ALT.	ent in EEG	EMQ Everyday Memory Questionnaire; MMSE Mini Mental State Examination; Log R Logical Memory (Paragraph recall) test; TMT Trail Making Test; COWAT Controlled Oral Word

Study – Journal – Year	H E grading	Nº of patients	Dose	Duration	Route of administration	Biohumoral effects	Neurophysiological effect	Neuropsyciatric effects
								Association Test; JLO Judgement of line orientation; DCT Digit Cancellation time; DCE Digit Cancellation errors

Two studies focused on a population with grade 4 as graded by the West Haven HE criteria: **the patients received 4 g /daily of ALC via intravenous route**. **All these studies demonstrated a significant decrease of serum ammonia and urea levels**. Serum ammonia levels and EEG score improved in patients treated with ALC versus placebo but in the study by Malaguarnera et al. (*2006*), the Glasgow score in ALC vs placebo is worsened, meanwhile in another study (Malaguarnera et al. *2008*) it appears improved in the group with ALC treatment. The low number of patients, the clinical heterogeneity, and diversity of precipitating factors did not allow us to draw appropriate conclusions.

HE, similar to other chronic conditions, compromises health-related quality of life with deep negative impacts on both physical and mental well being, and with negative social effects. Fatigue, depression, cognitive impairment, changes of personality, alterations in sleep patterns, cognitive and motor performance skills have been evaluated with various neuropsychometric tests. **ALC acts on a number of levels in the treatment of HE**. In fact ALC enhances the mitochondrial function, improves cerebral energy levels, protects against neurotoxic insults, improves thrombocytopoiesis, erythropoiesis, leucopoiesis and immune functions, plays major roles in the metabolism of carbohydrates and lipids, leading to an increase in ATP generation and in cell energy. Aside from being an essential component of fatty acid metabolism, A L C is also a free-radical scavenger and may contribute to the protection of cells against oxidative stress (Malaguarnera et al. *2011d*).

Conclusion

It is possible that "energy enhancing compounds (e.g. L-carnitine, ALC and creatine), that have shown to improve neurologic functions in chronic HE". ALC participates in cell volume and fluid balancing in all tissues that are affected by the tonicity of the extracellular environment (Peluso et al. *2000*). **Despite of 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** (Flanagan et al.*2010*). Hyperammonemia may also mitigate the brain edema in acute liver failure (Rama Rao and Norenberg*2012*). The beneficial effects of ALC, including modulation of cell energy production, fat metabolism, and immune function, as well as protection from mitochondrial, neurologic and cardiovascular damage may be useful in patients with H E. No significant signs of toxicity or side effects were reported.

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Competing interests

The author declares that he has no competing interests

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CHAPTER IV

Discussion

Current pharmacological treatment for HE

HE, as a syndrome, is characterized by many symptoms. These symptoms (discussed in the introduction) could change from patient to patient. In general, the terapy is symptomatologic and it is focused on decreasing precipitating factors and on the lowering of ammonia levels. It was demonstrated that precipitating factors are a diet with a large amount of protein and it is obligatory to manage the nutritional status of the patients The assessment of nutritional status in patients with cirrhosis is problematic. In addition, there are significant sex-related differences in body composition and in the characteristics of tissue loss, which limit the usefulness of techniques based on

measures of muscle mass and function in women. Techniques that combine subjective and objective variables provide reasonably accurate information and are recommended. Small meals evenly distributed throughout the day and a late-night snack of complex carbohydrates will help minimize protein utilization. Compliance is, however, likely to be a problem. Diets rich in vegetables and dairy protein may be beneficial and are therefore recommended, but tolerance varies considerably in relation to the nature of the staple diet (Amodio P 2013). In the past two decade the nutrional protocol contemplated protein restriction as a treatment, but it promotes protein degradation, decreases muscle mass, and can cause deterioration in the patient's status (Bemeur, et al. 2010). Many studies show a decrease in brached chain amino acid in HE patients. Branched chain amino acid (BCAA) supplements may be of value in the occasional patient intolerant of dietary protein. It was also assessed that a supplementation of these amino acid could decrease ammonia levels, though the mechanism of the beneficial effects of BCAA is unknown but there is evidence that it may stem from increased availability of substrates for protein synthesis in liver parenchyma (Morgan MY, et al. 2007).
Currently, treatment for HE is based on strategies aimed at reducing the concentration of circulating blood ammonia. One obvious strategy is to address the source of ammonia production, with the gut being a primary target. Reducing ammonia production will minimize its absorption into the systemic circulation and hence the brain's exposure to it (Rose CF, 2010).

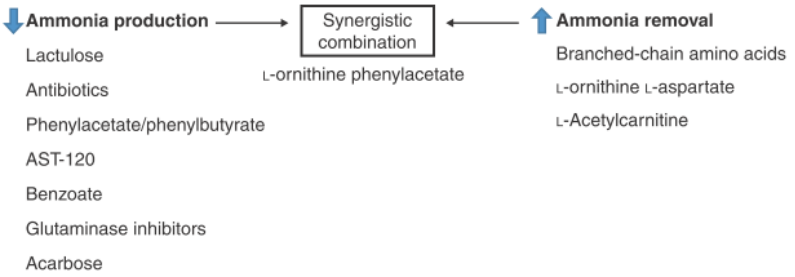

Figure 4. Ammonia lowering strategies (Rose CF 2010)

To decrease the ammonia production of the main strategies is using nonabsorbable disaccharides. In particular lactulose, is the first-line therapy for patients with end-stage liver disease and HE. Lactulose is an indigestible disaccharide, formed by glucose and fructose. It is metabolized by colonic bacteria in acetic acid and lactic acid. These acid decrease the gut pH suppressing the growth of the intestinal urease bacteria (ammoniagenic bacteria).

However, in 2004, a Cochrane review evaluating 22 clinical trials concluded that there was not enough convincing evidence arising from high-quality randomized trials to suggest that nonabsorbable disaccharides should be used to treat HE(Als-Nielsen B, et al. 2004). Lactulose is safe but it have not a good compliance. Infact lactulose treatment has been shown to cause abdominal cramping, bloating, nausea, vomiting, atulence, and abdominal distension, the last potentially leading to technical diffculties during surgery for liver transplantation. Moreover, lactulose treatment affects intestinal absorption, and this may amplify the nutritional deficits in patients.

Acarbose is a treatment for diabetes mellitus. Acarbose can decrease colonic proteolytic flora and dietary nitrogenous substances. It has demonstrated efficacy in treatment for overt hepatic encephalopathy.

Antibiotics - Orally administered antimicrobial agents targeting the gut have long been utilized with the primary aim of inhibiting urease-containing bacteria in the colon, thereby decreasing ammonia production and preventing absorption through the gastrointestinal tract. Antibiotics such as neomycin, metronidazole, vancomycin and rifaximin have all been demonstrated to lower blood ammonia. Among these antibiotic, Rifaximin demonstrated to be more efficient in the treatment of HE. Neomycin does not permit a long –term use for neurotoxicity, neprotoxicity and ototoxicity. Metronidazole is eliminitaed by the liver, this effect add a potential risk for a patient with a chronic liver disease. Vancomycin increase the risk of enteric bacterial resistance.

Rifaximin is a semisynthetic antibiotic that is poorly absorbed (<0.4%) and it has less adverse effect than neomycin, and its compliance is more than that offered by lactulose. Furthermore it decrease rapidly ammonia compared to these other compound. (Bass NM, 2010 ; Bajaj JS, et al. 2011). Rifaximin is able to modify bacterial metabolism and the pattern of metabolites, increasing serum fatty acids. It is also able to reduce peripheral inflammation. Probiotics reduce ammonia levels and improve performance in psychometric tests in patients with MHE (Mittal VV, et al. 2011).

Probiotics are better tolerated and have fewer adverse effects than lactulose and could be useful both for HE prophylaxis and treatment.

AST-120 is a microspherical carbon absorbent. It has demonstrated to have a good efficacy in the ammonia-lowering treatment both in patients (Pockros P, et al 2009) and in cirrhotic rats (Bosoi CR et al., 2009).

Sodium benzoate is administered to prevent glycine metabolism and thereby prevent the production of ammonia. sodium benzoate reduces blood ammonia levels and attenuates the symptoms of HE as effectively as lactulose, in cirrhotic patients. However, its effects are contrasting in literature because it has also been demonstrated that sodium benzoate can inhibit the production of urea, inducing hyperammonemia.

Sodium phenylbutyrate/phenilacetate. Sodium phenylbutyrate, which is rapidly oxidized into phenylacetate, is used to treat hyperammonemia by attenuating hyperglutaminemia in children with urea-cycle enzyme deficiencies. Phenylacetate conjugates with glutamine in the liver and kidney to form phenylacetylglutamine, which is incapable of being metabolized by glutaminase. The use of sodium phenylacetate/phenylbutyrate has never been investigated in the treatment of HE.

L -ornithine– L -aspartate. l-ornithine–l-aspartate (LOLA), both substrates of the urea cycle, were rst tried as infusion treatments in patients suering from end-stage liver disease and HE, in an attempt to lower blood ammonia by stimulating ureagenesis in the residual hepatocytes. In 2008, a Cochrane review, which included a meta-analysis, reported that LOLA

treatment led to the attenuation of OHE; it was, however, less benecial in patients with MHE.

Emerging therapeutic approaches

New therapeutic approaches acting on specific targets in the brain have also been described, and there are high hopes that they will improve cognitive and motor function in patients with MHE. Enhancement of cGMP levels in the cerebellum with phosphodiesterase 5 inhibitors has been shown to restore cognitive function in rats with MHE (Monfort P, et al. 2007) . These inhibitors are being used to treat erectile dysfunction in many cirrhotic patients and have not shown secondary adverse effects. Although one report suggests that these inhibitors may exacerbate portal hypertension and hyperdynamic circulation in patients with advanced cirrhosis and portopulmonary hypertension (Wang YW, et al 2006), they seem to hold promise for the improvement of cognitive impairment in patients with MHE (Felipo V, 2013). Antagonists of GABA A receptors (such as bicuculline) have also been shown to restore learning ability in rats with MHE (Cauli, et al. 2009). GABAergic tone may be selectively modulated by different types of compounds (for example, neurosteroids and benzodiazepines) acting on different receptor subtypes. Modulators that decrease GABA A receptor activation may induce anxiogenesis and convulsions, so appropriate modulators (and doses) must be found to improve cognitive function in MHE without adverse effects.

Targeting neuroinflammation may also be a useful strategy for treating MHE. Ibuprofen reduces neuroinflammation and restores learning ability and hypokinesia in rats with MHE (Cauli

2007a; 2009a). In cirrhotic patients, non-steroidal anti-inflammatory drugs are not recommended because they may induce secondary effects on the kidneys. Other types of anti-inflammatory drugs, such as p38 inhibitors, may be beneficial. Targeting microglial activation may reduce neuroinflammation without affecting the kidneys. p38 inhibitors reduce microglial activation and neuroinflammation and restore both cognitive and motor function in rats with MHE (El-Mlili, et al. 2008). Although p38 also has a key role in osmoregulation, at the moment, the main clinical application foreseen for p38 is to treat chronic inflammation. Several companies are developing p38 inhibitors to treat chronic inflammatory diseases (such as psoriasis and arthritis), which could be beneficial in MHE, but none of these drugs has yet been approved.

The most effective treatment for ALF is a liver transplant; however, it is not available for all patients. Two new promising approaches to delay cerebral damage in ALF are mild hypothermia (32–35 °C) and the administration of NMDA receptor antagonists. Mild hypothermia seems to attenuate most of the alterations that contribute to intracranial hypertension in animal models of ALF (Vaquero J 2012) and also in patients with ALF (Jalan R, et al. 2004) . Hypothermia reduces ICP by acting on different mechanisms that are believed to be important in its pathogenesis. Hypothermia reduces arterial ammonia concentration and its metabolism in the brain, and decreases cerebral blood flow, brain cytokine production and the levels of markers of oxidative stress (Jalan R, et al. 2004). Blocking NMDA receptors with antagonists substantially delays

death in rats with severe ALF and reduces mortality in those with milder forms of ALF (Cauli O, et al. 2008) . These procedures may increase survival, providing additional time to find a liver for transplantation or, in milder ALF, enable liver regeneration. Clinical trials to confirm their therapeutic utility are pending.

ALC treatment

Our studies treating HE patients with ALC exhibited recovery of neuropsychological activities related to attention, concentration, visual scanning and tracking, psychomotor speed, mental flexibility, short-term memory, attention and computing ability, language, orientation ability, cognitive activities. The ALC similar to structure to acetylcholine, exert a cholinomimetic effect. ALC is thought to influence the cholinergic system as a cholinergic receptor agonist and may promote the synthesis and the release of Acetylcholine. ALC has both antioxidant and antiapoptotic properties and can protect against various neurotoxic insults such as excessive glutamate (Forloni, et al. 1994), amyloid-beta exposure (Virmani, et al. 2001) and excessive ammonia concentration. The administration of ALC in compensated patients with cirrhosis could enhance the tolerance to protein load, low ammonia concentrations a, improve neurologic symptoms in patients with HE and reduce physical and mental fatigue. The decrease of ansiety and depression and the improvement in nutritional status and in physical activity lead to a positive spiral to further daily life activities (Bajaj, et al. 2009). Furthermore ammonia reduction improve neurocognitive activities in the treatment of severe HE.

Conclusion

Carnitine as a nutritional sumplement has been promoted as beneficial in a number of desorders of human carnitine deficiencies, suggesting that nutritional or pharmacologic supplements of carnitine might be beneficial in some disorders. Carnitine deficiencies has been associated with cirrhosis. There is a strong correlation between HE and abnormal ammonia handling and ALC has been shown to induce ureagenesis leading to decreased blood and brain ammonia levels, this is supported by other study that showed a protective effect of ALC against ammonia evoked encephalopathy in cirrhotic patients with ALC administration improving neuropsychological symptoms and plasmatic parameters in cirrhotic patients

During the work of these years, our group treated HE encephalopathy patients with different grade of HE with Acetyl-L-carnintine. Acetyl-L-Carnitine is a carnitine with acetylic group. Its properities are similar to the carnitine, blood but the acetylic group significantly increases blood brain barrier.

Two metaanalitic studies were conducted in these years taking in consideration the possible neuroprotective role of ALC in HE treatment. The oldest one assess that the main favorable effect of carnitine in improving ammonia concentration and NCT test is comparable to that of the current standard therapies of HE but the relatively low cost of this supplement could justify the use in the management of HE, whose treatment involves relatively high costs for public health . The authors of this article suggest as a future perspectives an increase in the dosage of ALC or the possible administration in combination with an antibiotic such as

rifaximin, that is currently used in United States for the treatment of HE (Shores NJ et al. 2008). The other study, confirm Shores et al. data judging the scientific article of this thesis as high quality paper using the Jadad score (Jadad et al. 1996). However, they suggest the utility of a Multicenter study to better assess the clinical efficacy of LAC in the treatment of HE (Quian Jiang, et al. 2013). ALC treatment reduce osmotic edema (Peluso 2000), decreae metabolic stress due to mitochondrial dysfunction and formation of reactive oxygen species (Hinerfeld, et al. 2004) and provide protection against neurotoxic agents

Additional studies are needed to better understand the molecular mechanisms underlying the clinical efficacy of LAC in the treatment of HE.

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