Emerson Lim
Columbia University Medical Center
Internal Medicine Residency Training Program
IRB Proposal

Title: Efficacy of zinc and L-carnitine in the treatment of hepatic encephalopathy

A. Study Purpose and Rationale

Hepatic encephalopathy (HE) is a complex neuropsychiatric complication in patients with advanced liver disease, with symptoms ranging widely from subtle findings only detected by psychometric testing (minimal hepatic encephalopathy) to stupor and coma. HE annually affects 30-45% of patients with cirrhosis. In 2002 about 35,000 patients were hospitalized in the United States with a diagnosis of HE costing $600 million, not including other medical costs or the cost of lost time from work or decreased workplace productivity.[1] And although the financial impact of minimal HE in patients has not been assessed, patients with this condition have reported impairment in their daily function in areas including social interaction and alertness, and have been found to have impaired abilities to safely drive automobiles.[2] As such, treatment to reduce the incidence of HE and minimal HE would likely reduce the impact on health care expenditure as well as significantly improve a patient's quality of life.

While impaired hepatic clearance of toxins is postulated as the cause of HE, the actual causative agent(s) and/or factors are still poorly understood. Currently, ammonia is thought to play a key role in hepatic encephalopathy with evidence that elevated levels alter properties of the blood-brain barrier, increase intracerebral edema by increasing intracellular glutamine, increase neuronal nitric oxide synthase expression, and act directly on GABA/benzodiazepine receptors. Other proposed substances thought to cause or contributed to hepatic encephalopathy include endogenously synthesized benzodiazepines or opioids, tryptophan, and manganese all of which would be cleared by otherwise healthy livers.[3]

Though the level of ammonia does not always correlate with a patient's mental status, current treatment of HE focuses on the reduction of ammonia levels by decreasing its production in the gut or facilitating its clearance. Standard therapy includes adherence to a protein-restricted diet (0.8g/kg/day) and the administration of lactulose, a disaccharide that serves as a cathartic, inhibits gut flora from producing ammonia, and also creates an acidic environment that facilitates movement of ammonia from the blood into the bowel lumen. These two forms of therapy are used for acute exacerbations of HE, as well as chronic management to prevent further episodes of HE. However, as the etiology of HE has not been fully elucidated, other adjunct therapies have been proposed and include using antibiotics against gut flora, branched chain amino-acids, benzodiazepine receptor antagonists, as well as zinc and L-carnitine.[4,5]

Previous studies have shown that L-carnitine decreases blood levels of ammonia and can be effective in the treatment of HE.[6] In cells, L-carnitine serves as a shuttle to pass acetyl-coA into mitochondria and is therefore important in the metabolic conversion of ammonia into urea. Zinc has also been implicated in the metabolism of ammonia to urea: two of the five enzymes in the urea cycle are zinc dependent. Additionally, as many patients with cirrhosis are deficient in zinc (attributed to poor intake, impaired absorption, or excess urinary loss), it is felt that zinc

supplementation may be beneficial in HE. Prior studies, however, have had contradictory results: one cohort study of 16 patients showed a significant effect of zinc supplementation on HE,[7] while an earlier double-blind crossover trial revealed no improvement.[8]

In addition to the standard therapy of protein restriction and lactulose administration some liver transplant specialists promote the use of both oral zinc and L-carnitine for the treatment of HE, while others feel that more data is needed before these additional drugs can be considered part of the standard of care. The purpose of this study is to determine the efficacy of zinc and L-carnitine together as an adjunct treatment for patients with HE.

B. Study Design and Statistical Analysis

The study proposed will be constructed as a double-blind, stratified, randomized, placebo-controlled trial involving four arms. Patients will be stratified based on their level of hepatic encephalopathy according to the West Haven criteria (Table 1).

Table 1. West Haven Criteria for Semi-quantitative Grading of Mental State[9]

Grade	Clinical Findings
1	Trivial lack of awareness, euphoria or anxiety, shortened attention span, impaired performance of addition
2	Lethargy or apathy, minimal disorientation for time or place, subtle personality change, inappropriate behavior, impaired performance of subtraction
3	Somnolence to semi-stupor, but responsive to verbal stimuli, confusion, gross disorientation
4	Coma

Patients with grade 3 or 4 HE will be excluded, and the remaining patients will be stratified as minimal HE (presence of at least one abnormal psychometric test), grade 1, or grade 2 HE. After stratification, patients will be randomly assigned via a computer generated model into four groups. All groups will receive the standard therapy of protein restriction and lactulose in addition to the proposed interventions. The first group will receive both zinc (200mg three times a day) and L-carnitine (1.5g twice a day), the second group will receive zinc (200mg three times daily) and a placebo twice daily, in the third group placebo to be taken three times daily and L-carnitine (1.5g twice daily), and the fourth group will receive 2 placebo pills to be taken three and two times daily. Based on prior studies, these four groups will be followed for a total of 90 days with evaluation by psychometric testing and blood analysis every 30 days. Compliance will be assessed by the percentage of pills returned.

Unpaired t-tests will be used to compare the different groups and as such was used as a basis for the power calculation (Chi squared analysis will be used for one secondary outcome point – the Child-Pugh score – and while it would be interesting if were to be significant, would not diminish the strength of the study if it were not significant. Chi squared analysis will thus not be used to determine the size of the study group). A prior study on the effect of L-carnitine on trail making tests showed an improvement in the time needed to connect numbered points on a piece of paper from 49 seconds to 27 seconds (effect ~22 seconds), with an average standard deviation of 20 seconds.[6] Given that zinc has not conclusively been shown to be effective in treating HE, it was assumed that zinc would only show half the effect of the L-carnitine group. Based on

these assumptions, 70 patients in each arm of the trial would be needed in order provide the study with a power of 80% at p=0.05.

The primary outcomes for this study will be performance on psychometric testing including timed trail making/number connecting tests (NCT), continuous reaction times to sound (CRT), as well as cognitive function tests including the block design test (BDT) and symbol digit modality test (SDMT). Secondary outcomes will include serum ammonia and zinc levels as well as calculated modified Child-Turcotte-Pugh (Table 2) and MELD scores, standardized methods used to predict mortality in patients with liver disease. Means and standard deviations will be calculated for each of the psychometric tests and will be compared via the unpaired t-test to assess significance of the data (set at $p<0.05$); categorical values such as the Child-Turcotte-Pugh score will be analyzed by Chi-squared testing. All patients will be maintained in their randomization groups for intention-to-treat analyses.

Table 2. Modified Child-Turcotte-Pugh Classification

	Points scored		
	1	**2**	**3**
Ascites	None	Easily controlled	Poorly controlled
Encephalopathy	None	Grade 1 or 2	Grade 3 or 4
Bilirubin (mg/dl)	<2	2-3	>3
Albumin (g/dl)	>3.5	2.8-3.5	<2.8
PT (sec > control)	<4	4-6	>6
	Classification		
	A	**B**	**C**
Total points	5-6	7-9	10-15

C. Study Procedure

Patients will be assessed at randomization then on a monthly basis for a total of three months at the CUMC and Cornell Liver clinics. There they will receive their routine care by their respective hepatologists in addition to undergoing psychometric testing. The trail making/number connecting test (NCT) and continuous reaction times to sound (CRT) are the most widely used psychometric tests and are used to evaluate abstract reasoning, tactile performance, spatial memory, intelligence, psychomotor speed, sequencing abilities, language function, sensory function and grip. The block design test (BDT) is used to assess construction, praxis, spatial reasoning, motor function, and processing speed, while the symbol digit modalities test (SDMT) evaluates attention, concentration executive and motor function, and processing speed.

In addition to these psychometric tests, routine lab work including a complete blood count, liver enzyme levels, and a coagulation panel to assess liver function will be taken at each visit. Additional blood work that would not require additional needle sticks would include analysis of ammonia and zinc levels.

D. Study Drugs
The first study drug is zinc sulfate, which will be administered orally. It is approved for zinc deficiency with a standard dosage of 200mg three times a day. It is generally well tolerated, but may cause dizziness, restlessness, diarrhea, nausea, vomiting, and gastric ulcers.

The second study drug is L-carnitine, which will also be administered orally during or following meals. It is approved for carnitine deficiency with a standard dosage of 1-3 grams/day. When taken by mouth, it is also well tolerated with side effects including diarrhea, nausea, cramps, and vomiting. More serious side effects include seizure, though the incidence is rare.

Patients will be monitored for side effects of these medications which will be tabulated and analyzed via Chi-squared testing. In the event that any serious side effect is noted, the study drug will be unblinded and discontinued, and the patient will be given the appropriate treatment.

E. Medical Device: none

F. Study Questionnaires: none

G. Study Subjects
Patients to be included in the study would need to be greater than 18 years of age, with either minimal hepatic encephalopathy or grade 1 or 2 HE based on the West Haven criteria, and documented cirrhosis of the liver diagnosed histologically or radiographically (either on ultrasound or CT).

Patients would be excluded from the study if they had major complications of portal hypertension such as variceal bleeding, hepatorenal syndrome, or spontaneous bacterial peritonitis. Other criteria for exclusion would be acute liver failure, concomitant neurologic disease, grade 3 or 4 HE, a known precipitant for hepatic encephalopathy including infection, fever, shock, and current drug use including alcohol, benzodiazepines, antidepressants, or stimulants. Once these conditions have resolved, patients may be considered for inclusion.

H. Recruitment of Subjects
Patients will be recruited at the New York-Presbyterian Hospital's Columbia and Cornell campuses as attendings and fellows on the Liver service see patients at both sites. Patients will be approached by residents, fellows, and attendings on both the in-patient and outpatient setting.

I. Confidentiality of Study Data
Confidentiality of patient data will be ensured by assigning each patient a code number through which their data can be accessed. Data will be stored in a secured location at CUMC accessible only to the investigators.

J. Potential Conflict of Interest: none

K. Location of the Study
CUMC and Cornell Liver clinics; an IRB approval will be obtained at the Cornell campus prior to starting trials there.

L. Potential Risks
The risk associated with this trial would only include the side effects related to the study drugs, zinc and L-carnitine. Though each has either been shown to improve or have little effect on hepatic encephalopathy, combining the medications may have other unforeseen effects and worsen HE, though the chance is unlikely as there has been no documented drug interactions between the two medications.

M. Potential Benefits
Patients may stand to benefit from the study by improving their mental status, resulting in an improved quality of life and decreasing their incidence of severe hepatic encephalopathy. Additionally, patients may be contributing to improvement in the standard of care for other patients with liver disease and hepatic encephalopathy.

N. Alternative Therapies
The study does not involve experimental drugs; however another potential therapy with evidence of efficacy is the use of antibiotics (rifaximin or neomycin). Other treatments that have had mixed results include the use of benzodiazepine antagonists (flumazenil) and increasing dietary intake of branch chain amino acid.

O. Compensation to Subjects
As the tests needed to be done at each clinic visit would only take an additional 5-10 minutes total, there will be no compensation offered to the subjects.

P. Cost to Subjects:
Study drugs and psychometric analysis will be provided to subjects of the study.

Q. Minors as Research Subjects: NA

R. Radiation or Radioactive Substances: NA

References:

1. Poordad FF. Review article: the burden of hepatic encephalopathy. *Alimentary Pharmacology and Therapeutics*. 2006;25(suppl 1):3-9.
2. Wein C, Koch H, Popp B. Oehler G, Schauder P. Minimal hepatic encephalopathy impairs fitness to drive. *Hepatology*. 2004; 39:739-45.
3. Ong JP, Mullen KD. Hepatic encephalopathy. *Eur J Gastroenterol Hepatol*. 2001;13:325-334.
4. Bass NM. Review article: the current pharmacological therapies for hepatic encephalopathy. *Alimentary Pharmacology and Therapeutics*. 2005; 25(suppl l):23-31.
5. Riodan SM, Williams R. Treatment of Hepatic Encephalopathy. *New England Journal of Medicine*. 1997;337(7):473-479.
6. Malaguarnera M, Pistone G, Elvira R, Leotta C, Scarpello L, Liborio R. Effects of L-carnitine in patients with hepatic encephalopathy. *World J Gastroentrol*. 2005;11(45):8197-7202.

7. Marchesini G, Fabbri A, Bianchi G, Brizi M, Zoli M. Zinc supplementation and amino acid-nitrogen metabolism in patients with advanced cirrhosis. *Hepatology*. 1996;23:1084-1092.
8. Riggio O, Ariosto F, Merli M, Caschera M, Zullo A, Balducci G, Ziparo V, Pedretti G, Fiaccadori F, Bottari E, et al.. Short term oral zinc supplementation does not improve chronic hepatic encephalopathy. Results of a double-blind crossover trial. *Dig Dis Sci*. 1991;36(9):1202-1208.
9. Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT, et al. Hepatic encephalopathy – definition, nomenclature, diagnosis, and quantification: final report of the Working Party at the 11th World Congress of Gastroenterology, Vienna, 1998. *Hepatology*. 2002;35:716-721.
10. Meyer T, Eshelman A, Abouljoud M. Neuropsychological changes in a large sample of liver transplant candidates. *Transplantation Proceedings*. 2006;38:3559-3560.