Efficacy of oral L-ornithine L-aspartate in cirrhotic patients with hyperammonemic hepatic encephalopathy

Claudia Isabel Blanco Vela,* Jorge Luis Poo Ramírez**


ABSTRACT

Hyperammonemia and associated cerebral edema cause neurological abnormalities in liver disease patients. Although only 15% of ammonia production originates in the colon, management strategies for hepatic encephalopathy (HE) have focused on reducing ammonia generation from the bowel rather than on manipulating systemic mechanisms involved in ammonia metabolism. Administration of L-ornithine L-aspartate (LOLA) improves mental status and decreases serum and spinal fluid ammonia levels by stimulating both the urea cycle and glutamine (Gln) synthesis, which are key metabolic pathways in ammonia detoxification. LOLA was shown to be superior to a placebo for management of HE, and the results of several clinical trials suggest that its effectiveness could be higher with the more severe grades of this syndrome. Compared with the standard treatment, LOLA is effective not only in reducing hyperammonemia and the severity of this disease, but also in improving the patient’s perceived quality of life. Therefore, LOLA is a promising alternative for the management of HE.


INTRODUCTION

Although the association between neurological abnormalities and liver disease was recognized by Hippocrates in the 5th century B.C., the pathophysiological mechanism responsible for these abnormalities remained obscure until the late 19th century. Liver dysfunction limits the metabolism of substances that are neurotoxic at high concentrations. The neurological manifestations of liver disease range from subtle changes in mental status to coma. This set of neurological changes is called HE.

HE and its association with decompensated liver disease is important because it has a negative impact on patient prognosis and quality of life, and treatment is costly. This syndrome affects up to 50% of cirrhotic patients with advanced disease. After the first episode of HE, the 1-year survival rate is 42% and the 3-year survival rate is 23%. In 2003, the total hospital cost associated with HE in the United States was estimated to be $900 million.

Ammonia is the neurotoxin that triggers HE syndrome. To date, treatment modalities have focused on reducing ammonia produced by the bowel rather than on manipulating mechanisms involved in the systemic production of ammonia.

Despite the limited number of clinical trials with high internal validity that support the use of nonabsorbable disaccharides (NADs) for treating HE, NADs are still considered the drugs of choice. LOLA decreases ammonia concentration by stimulating the urea cycle and expression of glutamine synthetase (GS). In cirrhotic patients, the synthesis of Gln via GS represents an alternative pathway for detoxification of ammonia.

PATHOGENESIS OF HEPATIC ENCEPHALOPATHY

For many years, it was thought that most of the ammonia in the body was produced by degradation of nitrogenous products by the colonic flora. However, current evidence indicates that 85% of the intestinal production of ammonia is the result of phosphate-activated glutaminase activity in the...
small intestine. The kidney also plays an important role in the generation of ammonia, and may contribute one-third or more of the ammonia released into the splanchnic circulation.

In healthy subjects, the main mechanisms involved in maintaining blood ammonia concentration within nontoxic limits are the urea production via the Krebs cycle and GS-mediated synthesis of Gln in the liver. In cirrhotic patients, a reduction in the hepatocellular function and the spontaneous generation of portosystemic shunts are responsible for hyperammonemia. In this situation, the processing of ammonia and its conversion into a nontoxic compound are carried out through Gln synthesis in the liver, brain and muscles.8,10

Ammonia passes freely through the blood-brain barrier (BBB), and is required for the production of Gln in astrocytes. The uptake of glutamate by the astrocyte and the production of Gln from glutamate and ammonia prevent excessive neuronal activation in healthy subjects. In liver disease, neurological abnormalities are associated with low-grade cerebral edema, which is secondary to hyperammonemia. The brain and spinal fluid of cirrhotic patients with hyperammonemia contain excessively high levels of Gln. Accumulation of ammonia and Gln in astrocytes results in oxidative stress, free radical formation and mitochondrial and sodium channel dysfunction, which ultimately increase intracellular osmolarity, causing edema and cerebral malfunction. Consequently, brain GS does not contribute to the detoxification of ammonia. However, the contribution of muscle GS to ammonia detoxification is substantial because this tissue constitutes a significant proportion of total body mass, and the Gln so produced is the major substrate for the generation of ammonia in the kidney. Hyperammonemia reduces the release of ammonia from the kidneys to the splanchnic circulation and increases urinary excretion of ammonia by up to 70%; that is, it becomes an ammonia-excreting organ and, therefore, is responsible for the systemic removal of this neurotoxin.

**MECHANISM OF ACTION OF L-ORNITHINE L-ASPARTATE**

Clinical studies supporting the use of LOLA in humans for treating HE began in Germany almost 40 years ago. LOLA is a salt of the natural amino acids ornithine and aspartic acid, and provides key substrates to metabolic pathways involved in the detoxification of ammonia. The administration of LOLA improves mental status and decreases ammonia levels in serum and spinal fluid by stimulating the urea cycle and the synthesis of Gln. After administration of LOLA, normalization of plasma ammonia levels is concomitant with a decrease in brain water content, which delays the onset of neurological symptoms.20

Ornithine stimulates the activity of carbamoyl phosphate synthetase I and aspartate stimulates the activity of arginase by donating nitrogen (Figure 1). Both of these enzymes are necessary for the synthesis of urea. The administration of LOLA decreases the plasma concentration of ammonia.
and increases the plasma concentration of urea, which proves that LOLA increases the activity of the Krebs cycle.\textsuperscript{21,22} When liver function is impaired, ammonia that cannot be metabolized by the liver is converted into Glu in the muscle. Thus, Glu functions as a nontoxic ammonia transporter in the circulation.\textsuperscript{7,9,10} After administration of LOLA, serum Glu levels increase because of the activity of muscle GS. However, levels of Glu and lactate in spinal fluid are not increased, which prevents the onset of cerebral edema. This supports that LOLA increases Glu synthesis in the periphery.\textsuperscript{20} The primary mechanism of detoxification of ammonia in cirrhotic patients is the uptake of ammonia that escapes the liver by muscle and its subsequent conversion to Glu in muscle.\textsuperscript{7,16} LOLA enhances the action of ornithine and aspartate transaminases to produce glutamate, which then promotes the synthesis of Glu by GS\textsuperscript{21} (Figure 2).

Ornithine passes through the BBB, suggesting that the central nervous system is a target for ornithine, but the mechanism by which it exerts effects is unknown. The improvement in mental status after therapy with LOLA is the result not of a direct effect of ornithine on the central nervous system, but of a decrease in exposure of the brain to ammonia secondary to a decrease in serum ammonia level.\textsuperscript{22}

**Efficacy**

As HE is diagnosed clinically, mental status is considered the primary outcome measure for establishing treatment efficacy in clinical trials. Objective clinical evaluation of HE is accomplished by analyzing the dominant frequency of an electroencephalogram, P300 auditory evoked potentials and the portosystemic encephalopathy index (PSEI). This index comprises electroencephalogram results, number connection test (NCT) results, the degree of asterixis, serum ammonia levels and the results of mental status evaluation. As minimal hepatic encephalopathy (MHE) has no apparent manifestations to clinical observers, the primary outcome measure is based on the results of neuropsychological and neurophysiological tests.\textsuperscript{23}

HE is in some cases episodic and resolved by removing the triggering event, making it essential that a drug show efficacy against a placebo before its effects are compared with those of an active control. The superiority of LOLA over placebo as an oral treatment for the management of HE has been demonstrated in human models.\textsuperscript{24-26}

The bioavailability of LOLA when administered p.o. is 82.2 ± 28\%,\textsuperscript{27} and the efficacy of its administration by this route was supported by Stauch, et al.\textsuperscript{25} This group of researchers compared the effectiveness of p.o. LOLA administered with a placebo in preventing hyperammonemia induced by a high-protein diet in 66 patients with chronic grade I or grade II HE or MHE according to the West Haven Criteria. Eighteen grams of LOLA or 10 g of fructose was given daily for 14 consecutive days. The primary outcome measures were postprandial ammonia concentration and NCT performance, and the secondary outcome measures were mental status and PSEI. LOLA was superior to the placebo in reducing postprandial ammonia level (p < 0.05) and in improving NCT performance (p < 0.01), mental status (p < 0.05) and PSEI (p < 0.01). Subgroup analysis showed that the most pronounced clinical response

![Figure 2. LOLA enhances the action of ornithine and aspartate transaminases in brain and peripheral tissues to produce glutamate, which promotes the synthesis of Glu by GS.](image-url)
to LOLA occurred in patients with grade II HE (57% of patients in LOLA group, whereas only 18% in the placebo group responded). In contrast, only 9% of patients with MHE showed a response to LOLA, suggesting that the more severe the impairment of mental status, the greater is the effect of LOLA. It is likely that the lack of response in the MHE group was a function of the method used for diagnosis of this condition. The use of a set of standardized neuropsychological tests or neuropsychological tests in conjunction with P300 or electroencephalogram dominant frequency tests, which are capable of detecting subtle neurological changes in the early stages of the disease, is recommended.

Kircheis, et al. compared the effect of intravenous LOLA with that of a placebo in 126 cirrhotic patients with hyperammonemia and MHE or grade I or II HE according to the West Haven Criteria. LOLA was superior to the placebo with respect to NCT performance (p < 0.001), postprandial ammonia level (p < 0.001), preprandial ammonia level (p < 0.01), mental status (p < 0.001) and PSEI (p < 0.01). The differences were more pronounced in patients with grade I or grade II HE than in patients with MHE, and, unlike Stauch, et al., they used both NCT and electroencephalography to diagnose MHE. This confirms the finding by Stauch that the greater the impairment in mental status, the greater is the effect of LOLA.

NADs are considered the drugs of first choice for treating HE. NADs act on the colon, shortening intestinal transit time and decreasing colon pH, which reduces the absorption of nonionized ammonia and increases the assimilation of ammonia by bacteria. As the evidence shows that only 15% of ammonia production originates from the colon, the contribution of NADs to the reduction of hyperammonemia is limited.

To compare the efficacy of oral LOLA with that of lactulose, 20 patients with grade I or grade II HE were randomized by Po, et al., to receive 30 mL of lactulose or 9 mg of LOLA orally for 2 weeks. The doses could be adjusted up to 60 mL of lactulose and up to 18 mg of LOLA, according to researcher’s opinion. The baseline ammonia concentration decreased from 120.4 ± 8.1 to 91.4 ± 10 µg/dL (p < 0.05) in the lactulose group, and from 141.6 ± 9.1 to 96.9 ± 9.3 µg/dL (p < 0.05) in the LOLA group. Although lactulose decreased baseline ammonia concentration, it did not affect the other variables used in the PSEI. In contrast, after administration of LOLA, a significant improvement in mental status, NCT, asterixis, and in the electroencephalogram activity, was observed (p < 0.05). The difference between PSEI values at baseline and those measured after 2 weeks in the LOLA group reach statistical significance (0.44 ± 0.03 and 0.28 ± 0.04, respectively; p < 0.05). Improvement in the quality of life was assessed using a EuroQol visual analog scale. The EuroQol index improved after both interventions (p < 0.05), the baseline and final indexes were 51.1 ± 24.1 and 61.5 ± 15.8, for the lactulose group and 56.5 ± 24.5 and 70 ± 19.4, for the LOLA group.

A meta-analysis designed to determine the efficacy and safety of LOLA for management of HE included the studies of Kircheis, Stauch, and Poo and analyzed data from 212 patients. According to this report, compared with the placebo, LOLA resulted in clinical improvement of HE (RR, 1.89; 95% CI, 1.32-2.71; p = 0.0005). Subgroup analysis showed that LOLA was superior to the placebo in grade I and grade II HE (RR = 1.87; 95% CI, 1.30-2.68; p < 0.0007), but had no significant effect in patients with MHE (RR = 1.69; 95% CI, 0.72-3.94; p = 0.23).

CONCLUSIONS

Currently, HE therapy is focused solely on reducing the amount of ammonia produced in the colon. However, evidence suggests that only 15% of ammonia of intestinal origin is produced in the colon. Therefore, there is no valid basis for using NADs as monotherapy for HE. LOLA promotes activation of the main ammonia detoxification routes and ammonia storage in the muscles as glutamine, a non-toxic carrier compound. Therefore, LOLA is involved in the systemic elimination of ammonia, making it an excellent therapeutic alternative.

The bioavailability of p.o. LOLA is 82.2 ± 28%, enabling it to be administered by this route without sacrificing efficacy. There is enough evidence that supports the superiority of p.o. LOLA over placebo for the management of HE. The studies of Stauch and Kircheis suggest that LOLA efficacy is greater in the more severe forms of the syndrome and warrants further examination of the effects of LOLA in grades III and IV of HE.

Controlled clinical trials comparing p.o. LOLA with standard therapy show that LOLA is as effective as NADs in the management of HE. The main impact of HE is not the treatment cost, but decreased survival and quality of life. The administration of LOLA has been proven effective, not only in reducing hyperammonemia and the severity of this disease, but also in improving the patient’s perceived quality of life.
ABBREVIATIONS

- HE: Hepatic encephalopathy.
- LOLA: L-ornithine L-aspartate.
- NADs: Nonabsorbable disaccharides.
- GS: Glutamine synthetase.
- Gln: Glutamine.
- PSEI: Portosystemic encephalopathy index.
- NCT: Number connection test.
- MHE: Minimal hepatic encephalopathy.

REFERENCES

A systematic review and meta-analysis of the use of oral zinc in the treatment of hepatic encephalopathy

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Abstract

Background and aim: Because low serum zinc levels precipitate hepatic encephalopathy, zinc supplementation is considered a potential therapeutic option. The aim of this study was to assess the effects of oral zinc supplementation in the treatment of hepatic encephalopathy.

Methods: For this systematic review and meta-analysis, data sources included electronic databases (CENTRAL, MEDLINE, EMBASE) and manual searching. Randomized clinical trials of adult patients diagnosed with liver cirrhosis and hepatic encephalopathy were included. The types of interventions considered were any oral zinc supplementation versus no intervention, placebo, or other interventions for the management of hepatic encephalopathy. The data were analyzed by calculating the RR for each trial and expressing the uncertainty as 95% CI. Continuous data were analyzed by calculating the standard mean differences (SMD) between groups within each trial and their 95% CI. Statistical heterogeneity was defined as a P-value > 0.10 (χ²) or I² > 25%.

Results: Four trials with a total of 233 patients were included. Oral zinc supplementation was associated with a significant improvement in performance on the number connection test (SMD –0.62; 95% CI –1.12 to –0.11) reported in three trials (n = 189), but not with encephalopathy recurrence reduction (RR 0.64; 95% CI 0.26 to 1.59) reported in two trials (n = 169). Other clinically significant outcomes (mortality, liver related morbidity, quality of life) were not reported.

Conclusion: Oral zinc supplementation improved performance on the number connection test, but no evidence about other clinical or biochemical outcomes was available.

Keywords: Therapy, Liver cirrhosis, Evidence-based medicine

Introduction

Hepatic encephalopathy is a neuropsychiatric complication of liver disease that affects 20 to 30% of the patients with cirrhosis [1,2], reducing health-related quality of life and causing a reversible decline in cognitive function. Previous studies have demonstrated that a reduction in blood ammonia levels improves hepatic encephalopathy, neuropsychological test performance, cognitive function, and health-related quality of life [3]. Lactulose, an ammonia absorption minimizer, has been successfully used to reduce blood ammonia levels in minimal hepatic encephalopathy. However, lactulose has no ammonia detoxification effect, rendering it ineffective to treat advanced hepatic encephalopathy [4-6].

Two major organs are involved in the metabolism of ammonia: the liver, in which ammonia is converted to urea via ornithine transcarbamylase, and the skeletal muscle, where ammonia is metabolized to glutamic acid via glutamine synthetase [5]. Zinc is a critical cofactor in these enzymatic reactions. Animal models have shown zinc deficiency decreases the activity of ornithine transcarbamylase, while zinc supplementation markedly increases hepatic ornithine transcarbamylase activity. Zinc deficiency has also been reported to impair the activity of muscle glutamine synthetase, which leads to hyperammonemia [6-8].
Zinc deficiency is observed frequently in patients with cirrhosis and hepatic encephalopathy [9]. Poor nutritional intake caused by a protein-restricted diet, impaired intestinal absorption, and excessive urinary loss are all potential causes of a low serum zinc levels in patients with advanced cirrhosis [5]. Short-term oral zinc supplementation may improve hepatic encephalopathy by correcting the zinc deficiency that compromises the conversion of ammonia to urea [10]. Bresci et al. reported better psychometric test performance in a zinc-supplemented group than in a standard therapy group, although the difference was not significant [11]. Similarly, oral zinc supplementation can improve hepatic encephalopathy in patients failing to respond to protein restriction and lactulose [2,6-8].

Zinc supplementation, in addition to standard therapies, may increase the hepatic conversion of amino acids into urea, decrease serum ammonia level, and consequently improve health-related quality of life. The effect of long-term oral zinc supplementation in addition to standard therapy on recurrent hepatic encephalopathy has not been established [7,8,12]. Despite the low cost and infrequent side effects of zinc supplementation, there is little evidence-based information about the effects of zinc supplementation on hepatic encephalopathy. The aim of this meta-analysis was to assess the effects of oral zinc supplementation in the treatment of hepatic encephalopathy.

Methods
Types of studies
Prospective randomized clinical trials that compared the effects of zinc supplementation with those of no intervention, placebo, or standard therapy on hepatic encephalopathy in patients with liver cirrhosis were included. Trials were included irrespective of publication status, year of publication, or language.

Types of participants
All adult patients diagnosed with liver cirrhosis using a combination of biochemical and clinical data, regardless of the etiology and treatment, diagnosed with hyperammonemia and hepatic encephalopathy were included.

Types of interventions
Studies that compared oral zinc supplementation with no intervention, placebo, or other interventions for the management of hepatic encephalopathy were included.

Types of outcome measures
The primary outcome measures were all-cause mortality, disease-specific mortality (mortality secondary to complications of liver cirrhosis), and severity of encephalopathy as assessed by performance on neuropsychometric tests or recurrence. The secondary outcome measures were adverse events (all types of adverse events) and quality of life score (measured by any scale).

Search methods for identification of studies
Electronic searches
Relevant randomized trials were identified by searching in CENTRAL, MEDLINE, and EMBASE.

Searching other resources
The references in all identified studies were inspected to identify other trials. The first or corresponding author of each included trial, as well as active researchers in the field were contacted for information about unpublished trials and additional information from their own trials.

Selection of studies
Two authors independently inspected each identified reference and applied the inclusion criteria. For potentially relevant articles or in cases of disagreement between the two reviewers, the full-text article was obtained and inspected independently; if the disagreement could not be solved, a third author reviewed the article. Justification for study exclusion was documented.

Data extraction and management
Two authors independently extracted the data from the included trials. In cases of disagreement, a third author extracted the data. Extracted data were discussed and this discussion was documented; when necessary, the authors of the original studies were contacted. Justification for study exclusion was documented. Trials were identified by the last name of the first author and the year of publication.

Assessment of risk of bias in included studies
Two authors independently assessed risk of bias in the trials without masking the trial names. Assessment was conducted according to the Cochrane Handbook for Systematic Reviews of Interventions [13].

Measures of treatment effect and data analysis
RevMan Analyses software was used for the statistical analysis [14]. Dichotomous data were synthesized and analyzed by calculating the RR and 95% CI for each trial. Continuous data were synthesized and analyzed by calculating the standard mean difference (SMD) between groups for each trial and its 95% CI.

Assessment of heterogeneity
We checked the heterogeneity of effects across trials by visual inspection of the forest plots and $\chi^2$ and $I^2$ tests for heterogeneity. Statistical heterogeneity was defined as $P > 0.10$ ($\chi^2$) or $I^2 > 25\%$. Whenever heterogeneity was detected,
subgroup analysis was performed to assess the effect of potential sources of heterogeneity on the main results.

Assessment of reporting biases
A funnel plot estimating the precision of trials (plot of logarithm of the RR against the sample size) was used to evaluate asymmetry and detect potential publication bias. In addition, Egger’s test was used to quantify the bias captured by the funnel plot [15].

Sensitivity analysis
We analyzed the data using both fixed and random-effect models. When both models produced similar estimates, the fixed-effect result was reported; otherwise, we reported the results from both analyses (Additional file 1: Figure S1). Outcomes were analyzed as reported in the trial, either per protocol or as an intention-to-treat.

Results
Study selection
A total of 65 potential references were retrieved: 36 were narrative reviews, 15 were nonrandomized studies, four were symposium reviews, one was a systematic review of different target trials, one was a clinical trial in animals, one was a trial in children, one was a clinical guideline, and one was a book chapter. Finally, five randomized controlled trials were included in the first analysis, but one study was excluded after a second evaluation of the inclusion criteria (Figure 1).

Study characteristics
We included four randomized controlled trials designed to evaluate oral zinc supplementation in the treatment of hepatic encephalopathy. The number of patients who received oral zinc supplementation ranged from 20 to 90. A total of 233 patients from three countries, Belgium [10], Italy [11], and Japan [1,16], were included. All studies involved patients with cirrhosis and different stages of encephalopathy. The doses used were zinc sulfate 600 mg/d [16], zinc acetate 600 mg/d [10,11], or polaprezinc 225 mg/d [1] (containing 51 mg of zinc and 174 mg of L-carnosine). All studies were randomized, double-blind, placebo-controlled trials (Table 1).

Risk of bias within studies
The risk of bias was unclear in all trials. Lack of information precluded a proper evaluation of the risk of bias for all studies.
Synthesis of results
Given the large heterogeneity of outcomes across studies, the meta-analysis was restricted to two primary outcomes: number connection test performance and rate of encephalopathy recurrence. Patients treated with oral zinc supplementation experienced a significant improvement in the number connection test performance (SMD \(-0.62; 95\%\ CI \(-1.12\) to \(-0.11\)) compared with patients in the placebo or no supplementation groups (Figure 2). Some heterogeneity of effects ($I^2 = 50\%$) was observed, and stratified analyses were conducted by year of the study and sample size, but no change in the direction or significance of the effect was observed (data not shown). The funnel plot shows no evidence of publication bias (Additional file 2: Figure S2). No reduction was observed in the encephalopathy recurrence rate (RR 0.64; 95\% CI 0.26 to 1.59) (Figure 3).

Reding et al. [10] studied the use of oral zinc supplementation in a double-blind randomized trial involving 22 patients with chronic encephalopathy. The zinc group received zinc acetate 600 mg/d. Compared to placebo, the zinc group showed improved performance in the number connection test (56 ± 25.4 and 42.12 ± 16.2 seconds, respectively).

Bresci et al. [11] assessed the effect of long-term zinc supplementation in 90 patients with cirrhosis with stable recurrent hepatic encephalopathy. Oral zinc supplementation (zinc acetate 600 mg/d) in addition to standard therapy normalized the serum zinc levels. Performance in the number connection test (40 ± 8 vs. 50 ± 12 seconds), as well as in the portal systemic encephalopathy index improved in the treated compared to placebo group (0.15 vs. 0.19). The treated group experienced less recurrence of encephalopathy; after six months 88.6\% of patients in the treated group had no detectable signs of hepatic encephalopathy, compared to 86\% in the placebo group.

Hayashi et al. [16] reported improved nitrogen metabolism in patients with liver cirrhosis after administration of branched-chain amino acids and zinc. Forty patients with liver cirrhosis, low serum albumin, and low zinc levels were randomized to receive either branched-chain amino acids alone or a combination of branched-chain amino acids and zinc supplements. Blood ammonia levels tended to increase in the amino acid group, while it decreased in the supplemented zinc group (post/pre change ratio of blood ammonia 1.22 ± 0.38 and 0.87 ± 0.26, $P = 0.003$, respectively). The Fischer ratio increased in both groups, but showed a sharper increase in the zinc-supplemented group.

### Table 1 Characteristics of trials included in this systematic review and meta-analysis

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<td>A: 72.6 ± 30.5/78.8 ± 27B: 141.6 ± 31.3/145.8 ± 30.4</td>
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<td>Polaprezinc 225 mg (containing 51 mg of zinc and 174 mg of L-carnosine)/d for 6 mo</td>
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<td>Effect of oral zinc on HRQOL and HE in patients with liver cirrhosis</td>
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ND No data, NCT Number connection test, HRQOL Health-related quality of life, HE Hepatic encephalopathy. *mean ± SD.
Takuma et al. [1] found that zinc supplementation was effective in treating hepatic encephalopathy and improving health-related quality of life (particular physical functioning, role-physical, and physical component scale). Seventy-nine patients with cirrhosis and hepatic encephalopathy were randomized to receive 225 mg of polaprezinc in addition to standard therapies. After six months zinc supplementation improved the Physical Component Scale score (P = 0.04) and the Child–Pugh score (7.8 ± 1.6 vs. 7.2 ± 1.4, P = 0.04), and significantly decreased hepatic encephalopathy grade (1.3 ± 0.9 vs. 0.9 ± 0.9, P = 0.03) and blood ammonia levels (112.0 μg/dL ± 56.3 vs. 90.4 μg/dL ± 33.4, P = 0.01). In this study one patient discontinued the treatment due to an adverse event (nausea and vomiting).

Discussion
In this meta-analysis, we included four randomized controlled trials evaluating the effect of oral zinc supplementation over hepatic encephalopathy. Three studies reported data on number connection test; all three showed an improvement in performance in the zinc group compared to placebo or standard therapy. This improvement suggests a beneficial effect of oral zinc in encephalopathy patients. Two studies reported data on encephalopathy recurrence rate. Both studies observed lower recurrence rates in the zinc groups, suggesting a beneficial effect of zinc; however, given the small sample size, confidence intervals were wide and failed to reach statistical significance.

Hepatic encephalopathy is characterized at the neurophysiological level by disturbed corticocortical and corticomuscular coupling, and at the cellular level by primary gliopathy [2,5,17,18]. Ammonia is a key pathophysiological factor in hepatic encephalopathy [18,19]. In the brain, ammonia is detoxified by astrocytes through a reaction catalyzed by glutamine synthetase; an increased brain glutamine/glutamate ratio is associated with decreased myoinositol, reflecting compensation for glial edema [20-23]. Swollen astrocytes predispose to neuronal dysfunction by impairing their regulatory activity against the increase in protein tyrosine nitration and the formation of reactive oxygen and nitrogen oxide species including nitric oxide. If not counteracted, these reactions promote RNA oxidation, which prompts gene expression and the transcription of altered proteins [2,5,6,18,19,21,24].

Cytokines or lipopolysaccharides could induce the formation of nitrogen oxide species and trigger zinc release from metallothioneins, the principal zinc storage protein. A fluctuation in intracellular zinc levels modulates signal transduction, transcription factor activity, and gene expression, causing hepatic encephalopathy symptoms. Zinc deficiency is associated with disturbances in learning, memory, and emotional stability and is accompanied by hyperammonemia. Zinc supplementation has shown to reduce ammonia levels in experimental animals and humans through hepatic urea synthesis stimulation and glutamine synthesis in skeletal muscle [2,6,12,18,19,21,25].

The present meta-analysis is limited by the small number and poor quality of trials included. Available trials studied heterogeneous outcomes and failed to measure critical outcomes such as quality of life. This hinders the ability to draw conclusions about the value of oral zinc supplementation in the treatment of hepatic encephalopathy. Additionally, little information regarding the clinical
importance of the different zinc formulations used in the trials was available.

In conclusion, oral zinc supplementation improved performance on the number connection test, but there is no clear evidence that supplementation improves encephalopathy or encephalopathy-related quality of life. More trials are needed to evaluate the use of oral zinc supplementation in patients with liver cirrhosis and hepatic encephalopathy.

**Additional files**

Additional file 1: Fixed model.
Additional file 2: Funnel plot.

**Competing interests**
The authors declare that no competing interests exist.

**Authors’ contributions**
NCC-T: protocol writing, searching, trial selection, data extraction, report writing, drafting the article, and final approval of the manuscript. AC-A: protocol writing, searching, trial selection, data extraction, report writing, drafting the article, and final approval of the manuscript. TB-G: report writing, drafting the article, and final approval of the manuscript. NM-S: report writing, drafting the article, and final approval of the manuscript. MU: report reading and approved the final manuscript.

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**References**


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