Applied nutritional investigation

Effect of zinc on liver cirrhosis with hyperammonemia:
A preliminary randomized, placebo-controlled double-blind trial

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ABSTRACT

Objective: To our knowledge, no randomized study has shown whether zinc replacement therapy is effective for hyperammonemia in liver cirrhosis; therefore, we performed a double-blind, placebo-controlled trial to examine efficacy and safety of the zinc replacement therapy.

Methods: Patients with liver cirrhosis and hyperammonemia (at or above the institutional reference value) and hypozincemia (<65 µg/dL) were enrolled in the outpatient units of the participating institutions and were randomly divided to receive placebo (P group) or zinc acetate preparation at a dose of 3 capsules/d for a total zinc content of 150 mg/d (Z group) by the envelope method. Of the 18 enrolled patients, 6 dropped out; thus, the analyses included 12 patients (5 in the P group and 7 in the Z group). Variations in blood concentrations of zinc and ammonia as well as liver function test results were compared.

Results: Blood zinc levels significantly increased in the Z group (P = 0.0037; Friedman test) but not the P group. Blood ammonia levels significantly decreased in the Z group (P = 0.0114; Friedman test) but not the P group. The percent change in blood ammonia level also revealed significant reduction at the eighth week in the Z group (P = 0.0188: Mann-Whitney test). No serious adverse events attributable to the zinc preparation were noted.

Conclusion: Although this study is preliminary and includes a small sample, it is to our knowledge, the first randomized controlled trial to show that zinc supplementation for 3 mo seems effective and safe for treating hyperammonemia in liver cirrhosis. Studies with a larger sample size are needed to confirm our findings.

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Introduction

Protein-energy malnutrition in patients with liver cirrhosis can degrade their prognoses for survival and cause complications such as hepatic encephalopathy [1–6]. The effectiveness of several nutritional interventions on nutritional and metabolic abnormalities associated with liver cirrhosis has been described. The administration of branched-chain amino acids improves protein metabolism, reducing the incidence of complications and improving prognosis for survival [7–9]. Additionally, consuming foods divided more than three times per day can improve abnormal energy metabolism [10]. Thus, the management of nutritional factors is clinically important when treating liver cirrhosis. Recently, among various nutrients, trace metals such as iron and zinc were found to be closely involved in the pathophysiology of liver cirrhosis [11–14]. To date, however, the mechanisms underlying their metabolism and pathologic significance have not been clarified.

Zinc plays an indispensable role in cell growth and differentiation and is very important for metabolism in humans [15]. More than 300 proteins contain domains with zinc, and these domains are important for regulating cellular functions [16–19]. Therefore, it is likely that zinc is closely involved in many bodily functions. The homeostasis of zinc in vivo is primarily preserved by a balance between the zinc-binding protein metallothionein and the expression of two zinc transporters [20–24]. If zinc stores become deficient, numerous problems, including growth disorder, cognitive disorder, and compromised immune function, can occur [25–28]. Zinc deficiency is likely to occur in patients with liver cirrhosis, and factors that are potentially responsible for such deficiency include disturbed zinc absorption by the digestive tract and increased zinc excretion in the urine [12,13]. Furthermore, diuretics, which are commonly used to treat edema and ascites, aggravate zinc deficiencies in patients with liver cirrhosis by increasing zinc excretion in the urine [29].

It has been suggested that zinc deficiency is related to the pathogenesis of hepatic encephalopathy. Several studies have shown a statistically significant inverse relationship between the serum levels of zinc and ammonia [30–32]. On the basis of these findings, several studies have examined the effects of zinc supplementation in patients with hyperammonemia [30,33–36]. Although two randomized controlled trials (RCTs) have been performed to examine these effects, the period of zinc supplementation in these trials was rather short (8 and 10 d), and the results were controversial [33,37]. One study showed that longer supplementation (3 mo) of zinc in patients with hepatic encephalopathy reduced serum ammonia levels and increased plasma urea levels, but this was not an RCT [14]. The main effects of zinc supplementation on ammonia metabolism proposed thus far are increased ammonia uptake of the muscle through activation of glutamine synthetase and increased activity of ornithine transcarbamylase, a key enzyme of the urea cycle in the liver [13]. Liver ornithine transcarbamylase activity was found to decrease in zinc-deficient rats, leading to increased plasma ammonia, whereas it significantly increased in zinc-supplemented cirrhotic rats compared with the control group [34].

In this study, we describe the results of a multicenter, placebo-controlled, double-blind randomized trial of zinc administration for 3 mo in patients with liver cirrhosis and hyperammonemia.

Methods

Participants

Between September 2009 and January 2012, patients who met the following criteria were enrolled at each institution: liver cirrhosis diagnosed by clinical symptoms, imaging studies, or histologic examination; blood ammonia level higher than the institutional reference value confirmed at least twice from blood samples collected within 2 mo before enrollment; hepatic encephalopathy grade ≤1 (grade 0, no symptoms; grade 1, the presence of euphoria or depression, mild confusion, sleepiness, or disordered sleep; grade 2, lethargy or moderate confusion; grade 3, marked confusion or sleeping almost all day; grade 4, coma) [38]; serum zinc concentration ≤65 μg/dL; age ≥20 y; and able to attend outpatient treatment. The exclusion criteria were as follows: hepatic encephalopathy grade ≥3; liver failure due to fulminant hepatitis; malignant disease requiring treatment during the period of the clinical trial; hospitalization required for cardiac, renal, or pancreatic disease; serious hematologic or cerebrovascular disorder; allergy to zinc preparations; and ineligibility for other reasons according to their attending doctors.

Protocol

The enrolled patients were randomly assigned to either the placebo (P) group or the zinc (Z) group by the envelope method, and the severity of hepatic insufficiency was thereafter diagnosed using hematologic tests including blood ammonia concentration and clinical symptoms, at 1-mo intervals for 3 mo. Patients assigned to the Z group took a single oral zinc acetate capsule (Nobelzin capsules, 50 mg: each capsule contains 167.84 mg zinc acetate dihydrate including 50 mg of zinc; Nobelpharma Co., Ltd., Tokyo, Japan) after each meal three times a day. Those assigned to the P group took one oral placebo capsule (identical to the zinc acetate capsule in color and form) three times a day. The adherence rates were ≥80% in all patients. In the safety assessment, adverse events (AEs) during treatment were observed at 1-mo intervals and severity was assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4).

Statistical analysis

The Friedman test was performed to determine whether there were changes at 1-mo intervals from the start to 3 mo after the start of treatment. The Mann–Whitney test was used to analyze the differences between groups before and at each time point during the study. The χ² test was performed to analyze the judgment by the attending doctors about treatment efficacy.

Clinical trial registration and ethical review

This clinical trial was registered at the University Hospital Medical Information Network in June 2009 (registration no. UMIN000002402). The trial was approved by the hospital’s ethical review board (no. 0990991063). The details of this trial were fully explained to each patient in oral and written form, and written consent was obtained.

Results

Analysis set

Eighteen patients who met the inclusion criteria at each institution were enrolled and assigned to either the P group (n = 8) or the Z group (n = 10) (Fig. 1). One patient in the P group dropped out of the study due to general fatigue and another due to development of liver cancer requiring treatment. These two patients were thus excluded from the analysis. Because the patient who developed liver cancer had received treatment for hepatocellular carcinoma before this trial, the liver cancer was assumed to be recurrence. In the Z group, one patient was admitted with bronchopneumonia and one patient developed dizziness and requested discontinuation of the trial despite mild symptoms. Treatment was discontinued in a third patient due to the aggravation of hepatic encephalopathy. These three patients were excluded from the analysis. Thus, the analysis ultimately included five patients in the P group and seven in the Z group. Patients’ baseline characteristics are shown in Table 1. Although no significant differences in age, sex, liver function, or
encephalopathy grade were observed between the two groups, the ratio of branched-chain amino acid to tyrosine was significantly higher in the P group than in the Z group.

**Blood zinc concentrations**

The Friedman test revealed significant treatment-associated changes in blood zinc concentration in the Z group but not in the P group. When blood zinc concentrations were compared between the groups, the levels were significantly higher in the Z group at weeks 4, 8, and 12 (Fig. 2).

**Blood ammonia concentrations**

Because the reference values varied among the institutions (upper limit of normal: 39–86 μg/dL), changes in blood ammonia concentrations after treatment were expressed as the percent change against the pretreatment (baseline) values (Fig. 3). The Friedman test revealed significant changes in relative blood ammonia concentrations in the Z group but not in the P group. Moreover, relative blood ammonia concentrations at week 8 were significantly lower in the Z group than in the P group.

**Other hematologic test results**

The Friedman test revealed no significant changes in blood levels of albumin, aspartate aminotransferase, alanine aminotransferase (Fig. 4A), total bilirubin, amylase, and creatinine, as well as in prothrombin time and BTR (Fig. 4B) in either group after treatment. An intergroup comparison at each time point by the Mann-Whitney test also revealed no significant differences.

**Adverse events**

Table 2 shows the AEs observed during the clinical trial. In the Z group, seven AEs were observed (four mild grade 1 AEs, two grade 2 AEs, and one grade 3 event). The patients who

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>P group (n = 5)</th>
<th>Z group (n = 7)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>73.6 ± 8.4</td>
<td>64.3 ± 7.1</td>
<td>0.1044</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>4/1</td>
<td>3/4</td>
<td>0.1248</td>
</tr>
<tr>
<td>BMI</td>
<td>24.2 ± 3.9</td>
<td>24.3 ± 4.9</td>
<td>0.8075</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>25.4 ± 10.3</td>
<td>34.3 ± 30.0</td>
<td>0.8075</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>41.6 ± 16.1</td>
<td>51.3 ± 24.2</td>
<td>0.6261</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>393.2 ± 88.6</td>
<td>394.4 ± 107.6</td>
<td>0.8075</td>
</tr>
<tr>
<td>T. bil (mg/dL)</td>
<td>1.48 ± 0.22</td>
<td>1.57 ± 0.57</td>
<td>0.4649</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.3 ± 0.4</td>
<td>3.5 ± 0.6</td>
<td>0.4168</td>
</tr>
<tr>
<td>PT (%)</td>
<td>71.4 ± 9.9</td>
<td>65.0 ± 12.9</td>
<td>0.3718</td>
</tr>
<tr>
<td>Amylase (IU/L)</td>
<td>119.5 ± 38.1</td>
<td>94.0 ± 47.4</td>
<td>0.3272</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>0.76 ± 0.22</td>
<td>0.70 ± 0.30</td>
<td>0.6261</td>
</tr>
<tr>
<td>Zn (μg/dL)</td>
<td>51.8 ± 8.3</td>
<td>55.1 ± 8.1</td>
<td>0.4168</td>
</tr>
<tr>
<td>BTR</td>
<td>4.3 ± 1.1</td>
<td>3.8 ± 3.2</td>
<td>0.0424</td>
</tr>
<tr>
<td>NH₃ (μg/dL)</td>
<td>104.6 ± 41.9</td>
<td>88.3 ± 43.6</td>
<td>0.4639</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BMI, body mass index; BTR, branched-chain amino acid to tyrosine ratio; Cr, creatinine; NH₃, ammonia; PT, prothrombin time; T. bil, total bilirubin; Zn, zinc.

* Values are expressed as mean ± SD.
† All P-values, except for sex, were calculated using the Mann-Whitney U test; the P-value of sex was calculated using the χ² test.
experienced aggravation of encephalopathy to grade 3 or pneumonitis of grade 2 were admitted for treatment and the zinc administration was immediately discontinued. Recovery was achieved in both patients. Zinc administration was discontinued at the request of a patient with grade 1 dizziness. In the patient with grade 2 diarrhea, symptoms were relieved after the zinc dose was reduced from three to two capsules a day. The other AEs spontaneously resolved when treatment was continued. In the P group, five AEs were noted, including one case of grade 3 general fatigue requiring hospitalization. Moreover, the other four grade 1 events also spontaneously resolved without need for treatment discontinuation.

**Judgment of treatment efficacy by the attending physicians**

According to a combination of clinical symptoms and test data, the treatment efficacy was judged as effective by attending physicians in five of the seven patients in the Z group but in none of the five patients in the P group (Table 3).

According to the \( \chi^2 \) test, the proportion of patients whose treatment was considered effective was significantly larger in the Z group than in the P group.

**Discussion**

It has long been known that patients with chronic liver disease, especially liver cirrhosis, are deficient in zinc, a status that may contribute to several clinical symptoms of liver cirrhosis, and could be relieved by zinc replacement [12–14,33–36]. However, although the effects of short-term (about 10 d) zinc supplementation have been studied via a double-blind randomized controlled method, the effects of long-term administration (>1 mo) have not yet been examined, and the evidence provided by other studies supporting the efficacy of zinc replacement is not very strong [12,13]. The significance of the present trial is that it is the first double-blind RCT to address the efficacy of zinc replacement therapy administered for 3 mo. The results of this trial demonstrated a significant increase in blood zinc concentrations in the Z group compared with the P group. Although blood ammonia concentrations decreased by \( \sim 30\% \) in the Z group, no significant changes were observed in the P group. The percent change in the levels was significant at week 8 of treatment in the Z group compared with the P group. These results show that zinc replacement therapy can be useful for reducing ammonia levels in patients with liver cirrhosis and zinc deficiency.

The obvious limitation of this trial is its small sample size. The previously reported rate of decreasing blood ammonia levels by zinc replacement therapy for liver cirrhosis was \( \sim 30\% \) [14,35]. Thus, we calculated the sample size required to detect such decrease between zinc preparation and placebo groups using the Power and Sample Size Calculation (Vanderbilt University, http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). Under the conditions of the SD of 0.2 in one group, \( \alpha \) error = 0.05, \( \beta \) error = 0.8, \( \delta \) = 0.3, \( \sigma \) = 0.2, and sample size ratio between groups = 1; 16 patients in two groups were required. This trial actually enrolled 18 patients in two groups, but the ultimate analysis set included 12 patients due to discontinuation and other reasons.

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**Fig. 2.** Serum zinc levels of patients in the Z group rose significantly (black solid line; \( P = 0.0037 \), Friedman test) during the trial, whereas those in the P group did not change significantly (dotted gray line; \( P = 0.4402 \), Friedman test). Intergroup differences were significant by Mann-Whitney test at weeks 4, 8, and 12.

**Fig. 3.** Relative and raw serum levels of ammonia. (A) Relative serum ammonia levels of patients in the Z group fell significantly (\( P = 0.0114 \), Friedman test), whereas those in the P group did not (\( P = 0.4717 \), Friedman test). The mean value in the Z group was significantly lower than that of the P group (Mann-Whitney test; \* \( P = 0.0188 \)) at week 8. (B) Raw serum levels of ammonia in the Z group significantly changed (\( P = 0.0014 \), Friedman test), whereas those in the P group did not change significantly (\( P = 0.4717 \), Friedman test). No significant difference in raw serum ammonia levels between groups was observed by the Mann-Whitney test at weeks 4, 8, and 12.
Although the sample size was insufficient, the blood ammonia levels in the Z group decreased by ~30% as anticipated. This finding supports that zinc replacement therapy is effective for relieving hyperammonemia in liver cirrhosis, a result that is consistent with several earlier reports [12–14, 33–36]. Moreover, no significant change in blood ammonia levels was observed in the P group during this trial. As a result, the difference between groups in the percent change in ammonia levels at week 8 reached statistical significance. It is not known whether the absence of significant differences at the other time points (weeks 4 and 12) is attributable to the low statistical power due to the small sample size, probable temporal changes in the effects of zinc on improving ammonia metabolism, or other reasons. Another possibility is that zinc supplementation needs some time to take effect (maybe >4 wk). Although an increase in serum zinc levels was clearly observed at week 4 post-zinc administration, it is possible that the administered zinc took some time to reach the cells and mitochondria and enhance enzyme activity. Future studies are needed that include larger patient groups and study periods >3 mo.

During this trial, seven AEs were observed in the Z group, including four grade 1 events, one grade 2 AE requiring hospitalization, and one grade 3 event. However, all of the patients who experienced AEs recovered. This trial targeted patients with decompensated liver cirrhosis accompanied by hyperammonemia, and one patient in the P group had an AE requiring hospitalization. The AEs observed in the Z group were consistent with complications of decompensated liver cirrhosis. Based on these facts, the two cases of AEs requiring hospitalization in the Z group likely were attributable to aggravation of the primary disease. However, because the AEs occurred during the clinical trial, it is undeniable that they might have been caused by the zinc preparation. On the other hand, the American Association for the Study of Liver Disease guidelines recommend zinc for treatment of presymptomatic Wilson’s disease or for maintenance therapy, because zinc not only has the ability to interfere with the uptake of copper from the gastrointestinal tract but also has very few side effects [39]. In a study with 141 patients with Wilson’s disease using the same zinc preparation (zinc content, 150 mg/day) as used in our trial for a maximum of 10 y and a mean of 4.8 y, it was reported that only transient stomach discomfort was observed as an AE in the early stage of treatment in 15% of the patients, but that no other problematic AE was seen [40]. In our trial, the observed AEs spontaneously resolved, except for the case of diarrhea, which was relieved by reducing the dose of the zinc acetate preparation. Taken together, these AEs are also likely to be associated with the primary disease. However, future studies are required to elucidate these issues.

### Table 2

<table>
<thead>
<tr>
<th>Event</th>
<th>Patients (n)</th>
<th>Grade*</th>
<th>Response</th>
<th>Recovery</th>
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<tbody>
<tr>
<td>Z group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>1</td>
<td>1</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Stomach ache</td>
<td>1</td>
<td>1</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1</td>
<td>1</td>
<td>Cessation</td>
<td>Yes</td>
</tr>
<tr>
<td>Pruritus</td>
<td>1</td>
<td>1</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Pneumonitis</td>
<td>1</td>
<td>2</td>
<td>Cessation</td>
<td>Yes</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>2</td>
<td>Dose</td>
<td>Yes</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>1</td>
<td>3</td>
<td>Reduction</td>
<td>Cessation</td>
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<tr>
<td>Worsening</td>
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<tr>
<td>P group</td>
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<td></td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>1</td>
<td>1</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>1</td>
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<td>No</td>
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<td>Dizziness</td>
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<tr>
<td>Constipation</td>
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<td>No</td>
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</tr>
<tr>
<td>General fatigue</td>
<td>1</td>
<td>3</td>
<td>Cessation</td>
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</tr>
</tbody>
</table>

* Grade was estimated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4).

### Conclusion

Although our results are only preliminary, this trial showed the effects of zinc supplementation for 3 mo on improving ammonia metabolism in patients with liver cirrhosis, and it presents promising data indicating the importance of RCTs with larger sample sizes and longer treatment durations for further elucidating the clinical efficacy of zinc preparations.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>P group</th>
<th>Z group</th>
<th>P-value</th>
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<tr>
<td>Effective</td>
<td>0</td>
<td>5</td>
<td>0.0133</td>
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<tr>
<td>Not effective</td>
<td>5</td>
<td>2</td>
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</table>
References