Effects of branched-chain amino acids and zinc-enriched nutrients on prognosticators in HCV-infected patients: A multicenter randomized controlled trial

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Abstract. Branched-chain amino acids (BCAAs) and trace element deficiencies are associated with poor prognosis in hepatitis C virus (HCV)-infected patients. The aim of this study was to investigate the effects of BCAA and zinc-enriched supplementation on prognostic factors in HCV-infected patients. Fifty-three HCV-infected patients were enrolled in this multicenter randomized controlled trial. The patients were assigned to either the placebo (n=27) or supplement group (n=26; 6,400 mg/day BCAAs and 10 mg/day zinc) and were followed up for 60 days. Primary outcomes were prognostic factors for chronic liver disease, including the serum BCAA-to-tyrosine ratio (BTR), zinc levels and α-fetoprotein (AFP) levels. There were no significant differences in any of the prognostic factors between the placebo and supplement groups at baseline. In the supplement group, the BTR and zinc levels were significantly increased compared with the placebo group (BTR: 5.14±1.59 vs. 4.23±1.14, P=0.0290; zinc: 76±11 vs. 68±11 μg/dl, P=0.0497). No significant differences were observed in AFP levels between the groups in the whole analysis. However, a stratification analysis showed a significant reduction in ΔAFP levels in the supplement group, with elevated AFP levels compared with the other groups (-2.72±3.45 ng/ml, P=0.0079). It was demonstrated that BCAA and zinc-enriched supplementation increased the BTR and zinc levels in the HCV-infected patients. Furthermore, the supplementation reduced the serum AFP levels in patients who had elevated serum AFP levels at baseline. Thus, BCAA and zinc-enriched supplementation may prolong the survival of HCV-infected patients by improving amino acid imbalance and zinc deficiency, and by partly downregulating AFP.

Introduction

The liver plays a central role in nutritional metabolism and, therefore, metabolic disorders, including amino acid imbalances and trace element deficiencies, occur frequently in patients with chronic liver disease (1). Branched-chain amino acids (BCAAs) are essential amino acids that play a crucial role in albumin synthesis and ammonia detoxification in patients with cirrhosis (2). In the clinical setting, reductions in serum BCAA levels can be assessed by measurement of the serum BCAA-to-tyrosine ratio (BTR) (1). Low serum BTR, along with low serum albumin levels, have recently been reported to be a predictive factor in the development of hepatocellular carcinoma (HCC), and confer a poor prognosis in patients with cirrhosis (3,4).

Zinc is a trace element that activates >300 metalloenzymes, including DNA polymerase (5). In addition, zinc stabilizes zinc finger proteins, which bind to DNA and modulate the transcription of target genes, including tumor

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Abbreviations: BCAAs, branched-chain amino acids; BTR, BCAA-to-tyrosine ratio; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOMA-IR, homeostasis model assessment for insulin resistance; AFP, α-fetoprotein, BMI, body mass index

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suppressor genes (6). In patients with chronic liver disease, serum zinc levels decrease as the severity of liver disease increases (7), and low serum zinc levels are known to be associated with the development of HCC and poor prognosis in hepatitis C virus (HCV)-infected patients (8,9). Thus, serum zinc levels are a significant metabolic prognosticator in HCV-infected patients.

BCAAs and zinc are pharmacological nutrients that exert diverse biological effects (1). A decreased risk of hepatocarcinogenesis and mortality has been reported in patients with chronic liver disease treated with BCAA (4,10,11) or zinc (8,9) supplementation. Therefore, a supplement containing both BCAAs and zinc may have the potential to improve prognostic factors in patients with chronic liver disease. Aminofeel® is a commercially available BCAA and zinc-enriched supplement. We previously examined the usefulness of the supplement and demonstrated that it improves insulin resistance, taste sensitivity and adherence to interferon therapy in HCV-infected patients (12-16). However, it remains unclear whether BCAA and zinc-enriched supplementation improves prognostic factors, including the platelet count, serum albumin levels, homeostasis model assessment for insulin resistance (HOMA-IR) value, serum BTR and zinc levels in HCV-infected patients. In addition, to the best of our knowledge, the impact of the supplement on serum α-fetoprotein (AFP) levels has never been investigated. Therefore, we performed a prospective multicenter randomized controlled trial to investigate the effects of a BCAA and zinc-enriched supplement on prognostic factors in HCV-infected patients.

Subjects and methods

Ethical considerations. This study was designed in 2009 by the steering committee of Research on Hepatitis, The Ministry of Health, Labour and Welfare of Japan (principal investigator, Michio Sata, MD), to evaluate the usefulness of BCAA and zinc supplementation in patients with chronic HCV infection. The study protocol was approved by the Ethical Committee of Human Experimentation in Kurume University School of Medicine (approval no. 09152, UMIN000012815), and is in accordance with the Helsinki Declaration of 1975, as revised in 1983. Written informed consent for participation in the study was obtained from each subject.

Subjects. HCV-infected patients aged 65 years or older who had serum albumin levels ≥3.5 and <4.0 g/dl were recruited for the study. The exclusion criteria were as follows: i) currently undergoing interferon therapy; ii) positivity for hepatitis B surface antigen or serum hepatitis B virus DNA; iii) presence of autoimmune hepatitis, alcoholic liver disease (ethanol consumption >50 g/day), primary biliary cirrhosis, primary sclerosing cholangitis, hemochromatosis or Wilson's disease; iv) presence of cardiac or renal disease or severe psychiatric disease; v) presence of HCC or within a year of treatment for HCC; vi) presence of esophageal or gastric varices at risk of rupture; vii) presence of diabetes mellitus with anti-diabetic medication; viii) history of consumption of the BCAA and zinc-enriched supplement; ix) having taken a BCAA-related medication or a BCAA-containing supplement within the preceding 90 days; and x) currently taking a trace element-related medication or a trace element-containing supplementation.

Study design and participants. We performed a prospective multicenter randomized controlled trial in an outpatient setting in six medical institutions in Japan. From 2010 to 2012, 54 HCV-infected patients who fulfilled the inclusion criteria were enrolled in the study. One patient withdrew consent for participation before randomization. The stratified randomization method was used to achieve balance among groups in terms of subjects' baseline characteristics. Randomization was performed centrally, and both patients and investigators were blinded to the patients' group assignment. The patients were allocated at a 1:1 ratio to the placebo (n=27) or supplement group (n=26). Two patients in the placebo group were withdrawn from the study due to withdrawal of consent (n=1) and anemia (n=1). Two patients in the supplement group were withdrawn due to general fatigue (n=1) and the appearance of a rash (n=1). Thus, four patients were excluded from the statistical analysis on day 60 owing to lack of data. Finally, 92.6% (25/27) of the patients in the placebo group (age, 74±5 years; female/male, 17/8) and 92.3% (24/26) of patients in the supplement group (age, 74±5 years, female/male, 15/9) completed the 60-day treatment period, and the efficacy and safety of the treatment were assessed (Fig. 1).

Intervention and assessment protocols. In the supplement group, the patients were given two sachets of the BCAA and zinc-enriched supplement containing 6,400 mg/day BCAAs and 10 mg/day zinc (Aminofeel, Seikatsu Bunkasya Co., Inc., Chiba, Japan), once after breakfast and again at bedtime (Fig. 2). In the placebo group, the patients were administered a sachet of placebo after breakfast and another at bedtime. Although the BCAAs, trace elements and vitamins were replaced with corn starch in the placebo (Table I), the appearance and taste of the placebo were similar to those of the supplement.

On days 0 and 60, we evaluated body mass index (BMI), subjective symptoms (fatigue, sleeplessness, muscle cramps, loss of appetite and taste disorders) using a visual analog scale (Fig. 2). A visual analog scale is a horizontal line, 100 mm in length, anchored by word descriptors for subjective symptoms at each end. The patients marked on the line the point that they felt represented their current subjective symptom. The visual analog scale score was determined by measuring the distance (in mm) from the left-hand end of the line to the point that the patient had marked, on which 0 mm indicates an absence of symptoms and 100 mm indicates the worst symptom.

The following blood biochemical parameters were measured in all the patients on days 0 and 60: White blood cell count, hemoglobin levels, platelet count, total protein levels, albumin levels, BTR, prothrombin time (international normalized ratio), ammonia levels, zinc levels, fasting blood glucose levels, hemoglobin A1c levels, fasting immune reactive insulin levels, HOMA-IR, total cholesterol levels, aspartate aminotransferase levels, alanine aminotransferase levels, γ-glutamyl transpeptidase levels, alkaline phosphatase levels, blood urea nitrogen levels, creatinine levels, AFP levels and HCV RNA levels. All the biochemical parameters were measured by standard clinical methods using venous blood samples

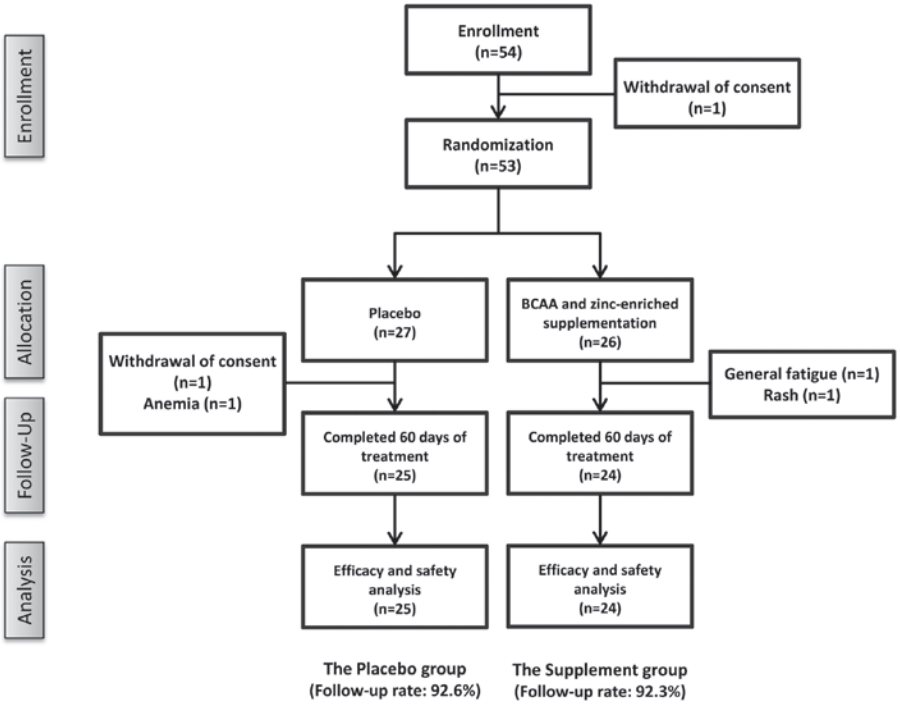

Figure 1. Flow diagram of the study. A total of 54 HCV-infected patients were enrolled. During the course of the study, one patient withdrew consent before randomization and four patients dropped out during the trial. In total, 49 patients completed 60 days of treatment/placebo, and the efficacy and safety of the treatment was assessed. The follow-up rates were 92.6% (25/27) in the placebo group and 92.3% (24/26) in the supplement group.

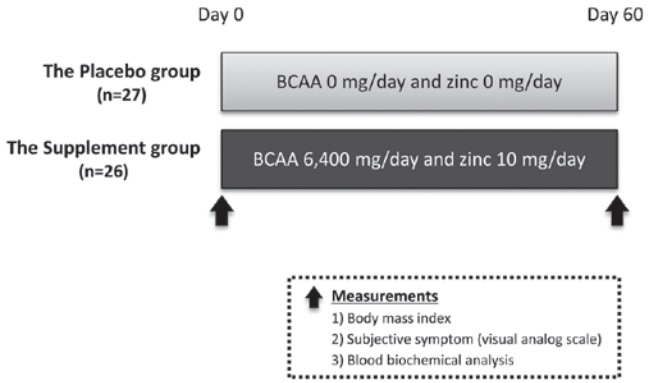

Figure 2. Intervention and assessment protocols used in the study.

taken the morning after a 12-h overnight fast, as previously described (17).

Outcomes. The primary outcomes were the following known prognostic factors for patients with chronic liver disease: Platelet count, serum albumin levels, serum AFP levels, HOMA-IR value, serum BTR and serum zinc levels. The secondary outcomes were BMI, subjective symptoms and biochemical examinations, including liver and renal functions, glucose metabolism and HCV RNA levels.

Stratification analysis according to serum AFP levels. As the serum AFP levels had a wide distribution, we performed a stratification analysis to assess the effects of the BCAA and zinc-enriched supplement on changes in serum AFP levels. The patients in both the placebo and supplement groups were further classified into two groups based on their serum AFP levels at baseline as follows: One group with AFP levels within the reference range (≤8.7 ng/ml) and another group with elevated serum AFP levels (>8.7 ng/ml). Changes in serum AFP levels were expressed as ΔAFP (day 60 AFP level vs. day 0 AFP level).

Safety monitoring. Safety was assessed in terms of vital signs, physical examination results, laboratory test results and clinical adverse events. When 19 patients (n=9 in the placebo group; n=10 in the supplement group) had completed the study, the data monitoring committee evaluated the safety and disadvantages of the groups and confirmed that there were no severe adverse events or disadvantages observed in either the placebo or supplement group. This evaluation was conducted to produce an interim report at the meeting of Research on Hepatitis (Ministry of Health, Labour and Welfare of Japan; principal investigator, Michio Sata).

Considerations relating to the sample size and treatment period. Based on the BTR data in our previous pilot study (12), the sample size was calculated using Power and Sample Size Calculation v 3.0, as previously described (18). Briefly, we planned to study a continuous response variable in independent controls and experimental subjects with one control per experimental subject. In our previous pilot study, the response within each subject group was normally distributed with a standard deviation of 0.9 (12). If the true difference between the experimental and control means is 1, we would need to study a total of 36 subjects (18 experimental subjects and 18 control subjects) to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with a probability (power) of 0.9. The type I error probability associated with testing this null hypothesis is 0.05.

Table I. Contents of one sachet of placebo and one sachet of the branch-chain amino acid and zinc-enriched supplement.

	Placebo	Supplement
Valine (mg)	0.0	800.0
Leucine (mg)	0.0	1,600.0
Isoleucine (mg)	0.0	800.0
Zinc (mg)	0.0	5.0
Calcium (mg)	0.0	21.1
Magnesium (mg)	0.0	12.6
Copper (mg)	0.0	0.2
Selenium (μg)	0.0	49.6
Chromium (μg)	0.0	14.4
Vitamin A (μg)	0.0	315.0
Vitamin D (μg)	0.0	3.0
Vitamin E (mg)	0.0	6.4
Vitamin K (μg)	0.0	29.6
Vitamin C (mg)	0.0	40.0
Vitamin B1 (mg)	0.0	2.4
Vitamin B2 (mg)	0.0	2.6
Niacin (mg)	0.0	12.0
Vitamin B6 (mg)	0.0	2.4
Vitamin B12 (μg)	0.0	10.0
Folic acid (μg)	0.0	0.2
Pantothenic acid (mg)	0.0	6.8
Corn starch (mg)	3,487.0	0.0

The treatment period was also based on BTR data from our previous pilot study (12). In the previous study, we examined the effects of the BCAA and zinc-enriched supplement on serum BTR 30, 60 and 90 days after treatment. Considering that the most significant efficacy was observed 60 days after treatment, a 60-day treatment period was used in this study (12).

Statistical analysis. All the data are expressed as the number or mean ± standard deviation. Differences between the placebo and supplement groups were analyzed using the χ^2 test or Mann-Whitney U test. Statistical comparisons between multiple groups were performed using the Kruskal-Wallis test. $P<0.05$ was considered to indicate a statistically significant difference. All analyses were performed using JMP 10.0.2 (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics. At baseline, there were no significant differences in age, gender and BMI between the placebo and supplement groups (Table II). No significant differences were observed in any subjective symptoms, including fatigue, sleeplessness, muscle cramps, loss of appetite and taste disorders between the two groups (Table II).

There were no significant differences in any of the prognostic factors, including platelet count, serum albumin and AFP levels, HOMA-IR value, serum BTR and serum zinc levels between the two groups (Table II). Moreover, no significant difference was observed in any of the biochemical examinations, including tests for liver and renal function, glucose metabolism and HCV RNA levels, between the groups at baseline (Table II).

Effects of BCAA and zinc-enriched supplementation on primary outcomes. On day 60, we evaluated the effects of the BCAA and zinc-enriched supplementation on the prognostic factors in the HCV-infected patients. There were no significant differences in platelet count, serum albumin and AFP levels, and HOMA-IR value between the placebo and supplement groups (Fig. 3A-D). Conversely, a significant increase in serum BTR was observed in the supplement group compared with the placebo group (Fig. 3E). In addition, a significant increase was observed in serum zinc levels in the supplement group compared with the placebo group (Fig. 3F).

Effects of the BCAA and zinc-enriched supplementation on secondary outcomes. On day 60, we also evaluated the effects of the BCAA and zinc-enriched supplement on BMI, subjective symptoms and biochemical parameters in the HCV-infected patients. There was no significant difference in BMI between the placebo and supplement groups (Table III). There was also no significant difference in fatigue, sleeplessness, muscle cramps, loss of appetite or taste disorders between the groups (Table III).

There was no significant difference in liver function test results, renal function test results, glucose metabolism or blood ammonia levels between the placebo and supplement groups (Table IV). In addition, no significant difference in HCV RNA levels was observed between the two groups (Table IV).

Stratification analysis according to serum AFP levels at baseline. As the serum AFP levels were widely distributed, a stratification analysis was performed according to the serum AFP levels at baseline to assess the effects of BCAA and zinc-enriched supplementation on changes in the serum AFP levels. A significant reduction in the ΔAFP levels was observed in the supplement group with an elevation in the AFP levels compared with the other groups (Fig. 4).

Safety. The incidence rates of adverse events during the study were 3.7% (1/27) and 7.7% (2/26) in the placebo and supplement groups, respectively. Two subjects discontinued the placebo due to withdrawal of consent (n=1) and anemia (Grade 2; n=1). Two subjects in the supplement group discontinued the treatment due to general fatigue (Grade 2; n=1) and rash (Grade 3; n=1). There was no significant deterioration in any biochemical parameters including liver and renal function. No Grade 4 or higher adverse events occurred during the study period.

Discussion

This was a multicenter randomized controlled trial designed to examine the effects of a BCAA and zinc-enriched supplement on prognostic factors in HCV-infected patients. No changes were observed in the platelet counts, serum albumin levels or HOMA-IR values; however, a significant increase was noted in the serum BTR and zinc levels in the patients administered

Table II. Patient characteristics at baseline.

	Placebo group	Supplement group	P-value
No. of patients	25	24	-
Age (years)	74±5	74±5	0.7480
Gender (female/male)	17/8	15/9	0.7688
Body mass index (kg/m^2)	22.9±3.7	22.6±3.3	0.7820
Subjective symptoms evaluated by visual analog scale (mm)			
Fatigue	20±21	21±22	0.9679
Sleeplessness	19±24	19±25	0.9272
Muscle cramp	14±25	20±29	0.7054
Loss of appetite	11±18	20±24	0.2278
Taste disorder	5±14	8±16	0.1828
Biochemical prognosticators			
Platelet count ($10^4/\mu$l)	13.6±5.8	14.2±4.4	0.4776
Albumin (g/dl)	3.79±0.12	3.79±0.20	0.6448
α-fetoprotein (ng/ml)	15.6±32.1	8.0±8.6	0.3372
HOMA-IR	2.99±3.81	2.89±2.38	0.7491
BCAA-to-tyrosine ratio	4.43±1.06	4.93±1.33	0.3731
Zinc (μg/dl)	68±9	68±7	0.8281
Other biochemical parameters			
White blood cell count (/μl)	4182±1092	4504±1132	0.3787
Total lymphocyte count (/μl)	1449±658	1515±826	0.7501
Hemoglobin (g/dl)	12.8±1.4	13.1±1.4	0.4007
Aspartate aminotransferase (U/l)	51±18	47±16	0.5283
Alanine aminotransferase (U/l)	47±23	37±20	0.0799
γ-glutamyl transpeptidase (U/l)	33±15	36±34	0.3650
Alkaline phosphatase (U/l)	340±132	318±113	0.6170
Total protein (g/dl)	7.55±0.63	7.44±0.62	0.5552
Prothrombin time (international normalized ratio)	1.05±0.12	1.02±0.06	0.9366
Fasting blood glucose (mg/dl)	102±12	109±23	0.3123
Hemoglobin Alc (%)	5.2±0.4	5.5±0.6	0.0932
Fasting immune reactive insulin (μU/ml)	11.0±10.8	10.1±6.4	0.8204
Total cholesterol (mg/dl)	160±23	158±28	0.6671
Ammonia (μg/dl)	32±13	30±16	0.5598
Blood urea nitrogen (mg/dl)	16.2±4.8	16.9±4.0	0.3916
Creatinine (mg/dl)	0.65±0.29	0.73±0.25	0.0872
HCV RNA (log copy/ml)	6.1±1.0	6.2±0.8	0.9544

The data are expressed as the number or mean ± SD. Differences between the placebo and supplement groups were analyzed using the χ^2 test or Mann-Whitney U test. $P<0.05$ was considered to indicate a statistically significant difference. HOMA-IR, homeostasis model assessment for insulin resistance; BCAAs, branched-chain amino acids; HCV, hepatitis C virus.

the supplement for 60 days. Furthermore, the stratification analysis revealed a significant reduction in the ΔAFP levels in the supplement group with an elevation in the AFP levels compared with the other groups.

No significant increase was observed in the serum albumin levels in this study. However, to the best of our knowledge, we demonstrated for the first time that 6,400 mg/day BCAAs administered for 60 days was sufficient to increase the serum BTR in patients in the early stages of HCV-related chronic liver disease. The dose of BCAAs used in this study may have been insufficient to increase serum albumin levels over the period of 60 days. However, a lower serum BTR can predict decreases in serum albumin levels (19), suggesting that long-term administration of the supplement may maintain constant serum albumin levels. In addition, low serum BTRs have been recently reported to be a predictive factor in the development of HCC, intrahepatic distant recurrence of HCC and poor prognosis in patients with cirrhosis (2,3). Moreover, BCAA supplementation is known to increase serum BTR, leading to the suppression of hepatocarcinogenesis and

Table III. Effects of the branch-chain amino acid and zinc-enriched supplement on body mass index and subjective symptoms.

	Placebo group	Supplement group	P-value
No. of patients	25	24	
Body mass index (kg/m^2)	23.2±3.6	22.8±3.2	0.7505
Subjective symptoms evaluated by a visual analog scale, mm			
Fatigue	18±17	18±25	0.5486
Sleeplessness	25±26	18±24	0.3095
Muscle cramp	16±23	23±33	0.5357
Loss of appetite	15±19	16±19	0.6210
Taste disorder	10±21	4±5	0.4153

The data are expressed as the number or mean ± SD. Differences between the placebo and supplement groups were analyzed using the Mann-Whitney U test. P<0.05 was considered to indicate a statistically significant difference.

Table IV. Effects of the branch-chain amino acid and zinc-enriched supplement on biochemical examinations.

	Placebo group	Supplement group	P-value
No. of patients	25	24	
White blood cell count (/μl)	4142±1010	4475±965	0.2711
Total lymphocyte count (/μl)	1494±650	1478±708	0.9915
Hemoglobin (g/dl)	13.1±1.4	13.0±2.0	0.8100
Aspartate aminotransferase (U/l)	52±17	54±21	0.9521
Alanine aminotransferase (U/l)	48±21	45±28	0.4064
γ-glutamyl transpeptidase (U/l)	34±16	37±40	0.2417
Alkaline phosphatase (U/l)	355±132	308±109	0.2846
Total protein (g/dl)	7.77±0.71	7.57±0.57	0.4412
Prothrombin time (international normalized ratio)	1.02±0.09	1.00±0.05	0.8915
Fasting blood glucose (mg/dl)	105±14	109±25	0.8886
Hemoglobin A1c (%)	5.2±0.4	5.6±0.6	0.0402
Fasting immune reactive insulin (μU/ml)	14.0±10.9	9.5±4.6	0.2155
Total cholesterol (mg/dl)	165±26	163±29	0.8258
Ammonia (μg/dl)	35±13	29±15	0.1906
Blood urea nitrogen (mg/dl)	14.9±4.5	16.9±4.5	0.0927
Creatinine (mg/dl)	0.64±0.27	0.72±0.25	0.0871
HCV RNA (log copy/ml)	5.9±1.5	6.1±0.9	0.7837

The data are expressed as the number or mean ± SD. Differences between the placebo and supplement groups were analyzed using the Mann-Whitney *U* test. P<0.05 was considered to indicate a statistically significant difference.

improvement in survival in patients with cirrhosis (4,10,11). Taken together, it would be worthwhile to test the effect of the long-term administration of the supplement on hepatocarcinogenesis and prognosis in HCV-infected patients.

We also demonstrated that the administration of 10 mg/day zinc was sufficient to increase the serum zinc levels in patients in the early stages of HCV-related chronic liver disease. In patients with cirrhosis, zinc upregulates hepatic ornithine transcarbamylase activity, a key enzyme in the urea cycle, and enhances hepatic ammonia detoxification (20). A recent meta-analysis by Chavez-Tapia *et al* (21) demonstrated that oral zinc supplementation improved performance in the number connection test, a test for hepatic encephalopathy. Thus, the supplement tested here may be beneficial for patients with hyperammonemia. In addition, a decrease in serum zinc levels is known to predict the development of HCC and poor prognosis in HCV-infected patients (8,9). Moreover, decreased risks of hepatocarcinogenesis and mortality have been observed in HCV-infected patients with increased serum zinc levels owing to zinc supplementation (8,9). The data reported here indicate that the zinc-enriched supplement has the potential to suppress the onset of HCC and improve prognosis in HCV-infected patients.

When all of the data were analyzed, the serum AFP levels were not significantly decreased in the patients administered

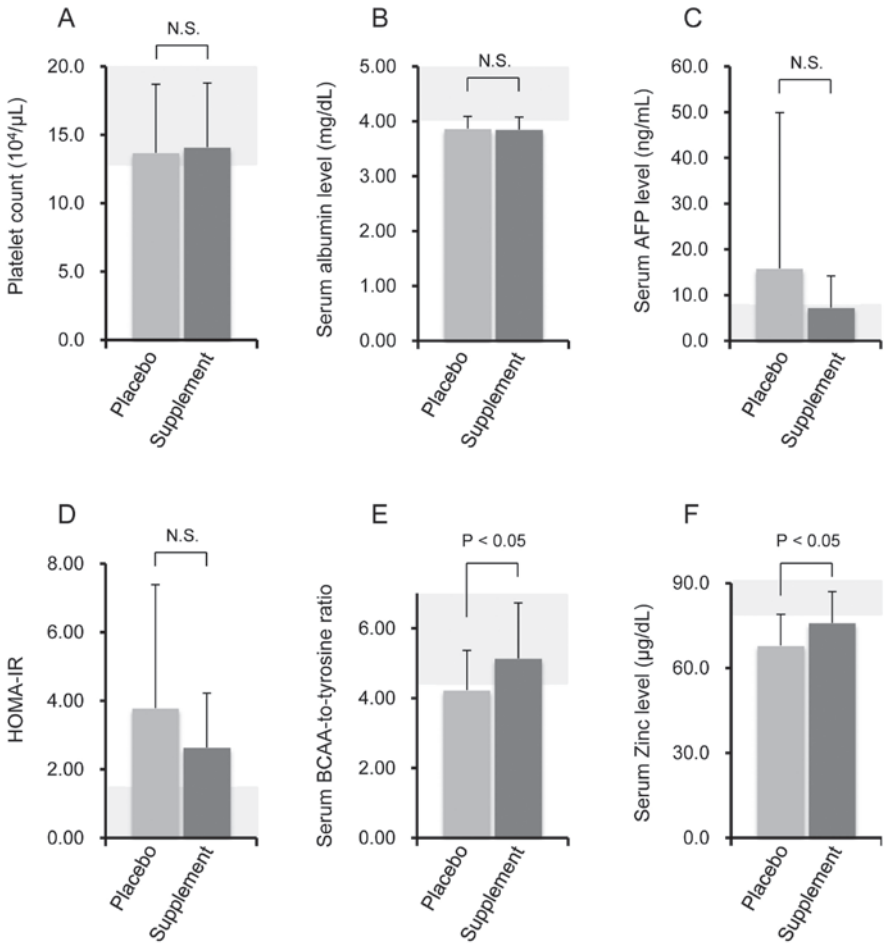

Figure 3. Effects of the BCAA and zinc-enriched supplement on prognostic factors: (A) Platelet count, (B) serum albumin level, (C) serum AFP level, (D) HOMA-IR value, (E) serum BCAA-to-tyrosine ratio and (F) serum zinc level. The data are expressed as the mean ± SD. The gray area is within the reference values of each parameter. Differences between the placebo and supplement groups were analyzed using the Mann-Whitney U test. P<0.05 was considered to indicate a statistically significant difference. AFP, α-fetoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; BCAA, branched-chain amino acids.

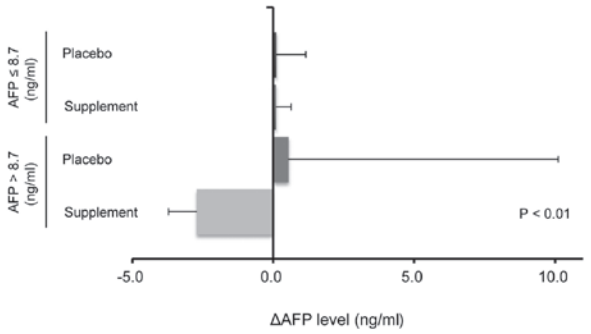

Figure 4. Stratification analysis according to the serum α-fetoprotein (AFP) level at baseline. The patients in the placebo and supplement group were further classified into two groups based on the serum AFP level at baseline: One group with AFP levels within the reference value (≤8.7 ng/ml) and another group with elevated serum AFP levels (>8.7 ng/ml). Changes in the serum AFP levels were expressed as ΔAFP (day 60 AFP level vs. day 0 AFP level) and compared among the groups. Statistical comparisons between multiple groups were performed using the Kruskal-Wallis test. P<0.05 was considered to indicate a statistically significant difference.

the BCAA and zinc-enriched supplement. However, the stratification analysis revealed a significant reduction in the ΔAFP levels in the supplement group with elevated AFP levels at baseline compared with the other groups. Although the reasons for the supplement-induced reductions in serum AFP levels are unclear, our findings are supported by previously published studies. First, Hagiwara *et al* (22) reported that BCAAs induce apoptosis in HCC cell lines by promoting a negative feedback loop from the mammalian target of rapamycin complex 1/S6K1 to the PI3K/Akt pathway and by suppressing the mammalian target of rapamycin complex 2 kinase activity towards Akt. Second, zinc stabilizes zinc finger proteins, which bind to DNA, and Nakao *et al*, as well as Xie *et al*, reported that zinc fingers and homeoboxes 2 and zinc finger and BTB domain-containing protein 20 repress the postnatal expression of AFP by interacting with the AFP gene promoter regions (23,24). Thus, BCAAs and zinc may independently contribute to a reduction in serum AFP levels by causing apoptosis of hepatoma cells and repressing AFP expression.

In this study, the BCAA and zinc-enriched supplement did not affect the platelet count, HOMA-IR value or HCV RNA levels. Conversely, previous basic studies demonstrated that valine, a BCAA, increased blood platelet counts in carbon tetrachloride-treated cirrhotic rats (25). Leucine and isoleucine have been shown to improve insulin resistance in mice fed a high-fat diet (26,27). Valine has been shown to suppress HCV genome replication in a dose-dependent

manner (28). Although the reason for the discrepancy between these previous studies and our study remains unknown, BCAAs may exert beneficial effects on the platelet count, HOMA-IR value and serum HCV RNA levels only under specific conditions. We also demonstrated that no subjective symptoms were significantly improved by the BCAA and zinc-enriched supplementation. BCAAs and zinc have been previously reported to improve muscle cramps and taste disorders (16,29,30), respectively. However, these symptoms were mild in the study subjects at baseline. This may explain why significant changes in muscle cramps and taste disorders were not evident in this study.

In conclusion, we examined the effects of a BCAA and zinc-enriched supplement on prognostic factors in HCV-infected patients. There were no significant changes in platelet count, serum albumin levels or HOMA-IR values. However, serum BTR and zinc levels were significantly improved by the supplementation. In addition, a stratification analysis revealed a significant reduction in ΔAFP levels in the supplement group, with an increase in AFP levels compared with the other groups. In light of these results, we conclude that the BCAA and zinc-enriched supplement may improve prognosis in HCV-infected patients by improving amino acid imbalance, reducing zinc deficiencies and partly downregulating AFP expression.

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