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Inhibitory effect of branched-chain amino acid granules on progression of compensated liver cirrhosis due to hepatitis C virus

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Background. A phase II randomized controlled trial was conducted in patients with compensated liver cirrhosis to investigate the inhibitory effect of branched-chain amino acid (BCAA) granules for oral use (TK-98) on disease progression. ***Methods.*** Patients who had compensated liver cirrhosis due to hepatitis C virus with baseline serum albumin levels between 3.6 and 4.5 g/dl were assigned to the TK-98 group, which was treated with BCAA granules (TK-98) for 168 weeks, or to a control group (no treatment). ***Results.*** No symptoms indicating decompensated cirrhosis, including ascites, edema, and hepatic encephalopathy were reported in either the TK-98 or control group during the study observation period. Hepatocellular carcinoma (HCC) was noted in eight of the 39 patients studied, and of these three received TK-98 (15.8%) and five were untreated (25.0%). A time-to-event analysis for the effect of BCAA therapy on development of HCC revealed no statistically significant differences between the two groups. However, an additional analysis of data from a subgroup with a baseline serum albumin level of <4.0 g/dl showed that the incidence of HCC was likely to be lower in BCAA-treated patients. ***Conclusions.*** BCAA may inhibit hepatic carcinogenesis in patients with compensated cirrhosis with a serum albumin level of <4.0 g/dl.

Key words: BCAA, HCV, compensated liver cirrhosis, hepatocellular carcinoma

Introduction

Liver cirrhosis is classified into two types according to the progression phase of the disease: compensated cirrhosis and decompensated cirrhosis. For improved prognosis and quality of life of patients with liver cirrhosis, it is important to delay progression of the disease from the asymptomatic compensated phase to the decompensated phase, which is accompanied by symptoms such as ascites, edema, and hepatic encephalopathy. The use of branched-chain amino acid (BCAA) granules has been shown to improve hypoalbuminemia in decompensated patients with cirrhosis and hypoalbuminemia despite adequate dietary intake. In addition, several studies have reported that BCAA granules improve the above symptoms of decompensated cirrhosis as well as delay development of serious complications that affect the prognosis for survival.[1–5] Therefore, the drug has now been extensively used for the purpose of improving serum albumin levels and ameliorating the disease state in patients with cirrhosis.

Serum albumin levels have been reported to serve as an important indicator of the severity of liver cirrhosis, and the maintenance or improvement of these levels is vital for improving the prognosis of liver cirrhosis.[3] We conducted a phase II randomized controlled trial to investigate whether supplementation with BCAA granules increased lowered serum albumin levels and delayed progression of the disease in patients with compensated cirrhosis. Furthermore, we also explored the inhibitory effect of BCAA therapy on development of hepatocellular carcinoma (HCC), based on results of a study showing that the development of HCC has a substantial impact on prognosis of patients with cirrhosis and that the lower the serum albumin level, the greater the risk of HCC.[6]

Materials and methods

Study design

This study was conducted in accordance with Japanese Good Clinical Practice, after review and approval by the

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Institutional Review Board of Toranomon Hospital. Subjects were fully informed of the nature of the study, and informed consent to participation in the study was obtained in writing from each subject. Patients enrolled were randomized to receive either BCAA granules (TK-98) or no treatment (control).

The inclusion criteria were as follows: (1) presence of compensated cirrhosis due to hepatitis C virus; (2) no prior or concurrent ascites, edema, or hepatic encephalopathy; (3) serum albumin level between 3.6 and 4.5 g/dl within 2 months prior to the study; (4) male sex and age between 50 and 70 years inclusive. Excluded from the study were patients who had or were considered to have HCC or cancer other than HCC, those with concurrent alcoholic cirrhosis and alcohol dependence, and those receiving nutritional supplements for the management of hepatic failure.

As the present study was intended to evaluate the effect of BCAA, study subjects were those with hepatitis C virus (HCV)-related cirrhosis. Such patients account for more than 60% of Japanese patients with liver cirrhosis.[7] The study had as an additional objective the exploration of the inhibitory effect of BCAA on HCC; therefore, the inclusion criteria included male sex and age between 50 and 70 years, because men in that age range are generally considered to have a propensity to develop HCC.[8]

The following drugs were prohibited during the study: high-BCAA agents for treatment of hepatic disorders, because these may alter plasma albumin and malotilate levels. There were no other restrictions on the concomitant use of drugs.

The primary end point was time to onset of ascites, edema, or hepatic encephalopathy, which are considered to be an indication of disease progression to decompensated cirrhosis. Transition to the decompensated phase of cirrhosis was defined to the time point at which one of the following manifestations was noted for the first time: (a) ascites found on palpation, (b) slight edema in the lower extremities, and (c) grade I or higher hepatic encephalopathy. The secondary variables were serum albumin level, blood Fischer's ratio (BCAA/aromatic amino acids, molar ratio), development of jaundice, performance status (PS), and development of HCC.

It has been reported that the serum albumin level decreases at a rate of 0.15 g/dl per year in patients with liver cirrhosis.[9] We assumed that a serum albumin level above an approximate threshold of 3.5 g/dl might indicate transition to decompensated cirrhosis.[10] Therefore, patients enrolled in the study were expected to have a baseline serum albumin level between 3.6 and 4.5 g/dl. We made the assumption that 15% of the control group would progress to decompensated cirrhosis annually and that treatment with TK-98 would reduce the progression rate to 5% with a hazard ratio of around 3.2. An observation period of 168 weeks was chosen on the presumption that compensated cirrhosis might progress into the decompensated phase in around 3.5 years in half of the patients. For a statistical significance level set at two-sided 20% and a statistical power at 60%, the sample size needed for the analysis was calculated to be 17 patients per group. Estimating a dropout rate of 15%, we set the target number of study patients at 20 patients per group, that is, a total of 40 patients.

Study checkups were carried out at 8-week intervals for the presence or absence of ascites, edema, hepatic encephalopathy, or jaundice; PS; subjective and/or objective symptoms; and laboratory parameters. In addition, each study subject was assessed for development of HCC with diagnostic imaging at intervals of 24 weeks. When any abnormal changes were noted in serum α-fetoprotein or protein induced by vitamin K absence or antagonist II levels, examination for HCC was additionally undertaken as appropriate.

The TK-98 group and control group each consisted of 20 subjects. Patients were dropped from the study if any symptoms of ascites, edema, hepatic encephalopathy, or jaundice appeared, indicating the decompensated phase of cirrhosis, or if HCC was found to have developed during the study period.

Study drug

BCAA granules (TK-98) containing 952 mg of L-isoleucine, 1904 mg of L-leucine and 1144 mg of L-valine per packet were orally administered to subjects at doses of one packet three times daily after meals. The control patients received no treatment.

Statistical analysis

Statistical analysis was performed with SAS Release 9.1.3 Service Pack 2. A time-to-event analysis was carried out to determine the transition to the decompensated phase of cirrhosis using the time point of event onset at which any of symptoms such as ascites, edema, or hepatic encephalopathy were noted for the first time. Survival functions were estimated by the Kaplan-Meier method, and the survival functions were compared between the two groups by using the log-rank test. Cox's proportional hazards models were used to examine the effect of the treatment and prognostic variables. Serum albumin levels and Fischer's ratio data were analyzed by using a mixed-effects model in terms of respective time-course patterns.

Results

Disposition of patients

Study subjects were selected from patients with compensated cirrhosis who visited the Department of Hepatology, Toranomon Hospital between January 1999 and March 2003. A total of 40 patients who met the inclusion criteria and gave written informed consent were enrolled in this study. Flow chart of patients through the trial is shown in Fig. 1. Of these 40 patients, one was dropped from the study prior to study commencement because he withdrew his consent, and nine patients were dropped from the study during the study period because of the development of HCC in eight patients and for a visit-related reason in the case of the remaining patient. All 39 patients who began the study were judged to be eligible and were included in the full analysis set and the per protocol set, as well as in the safety analysis.

Patient demographic and baseline characteristics are shown in Table 1. No significant differences were noted between the two groups with respect to age, concurrent esophageal varices or diabetes mellitus, history of alcohol drinking, serum albumin levels, blood Fischer's ratio, total bilirubin, platelet count, serum aspartate aminotransferase levels, or serum alanine aminotransferase levels.

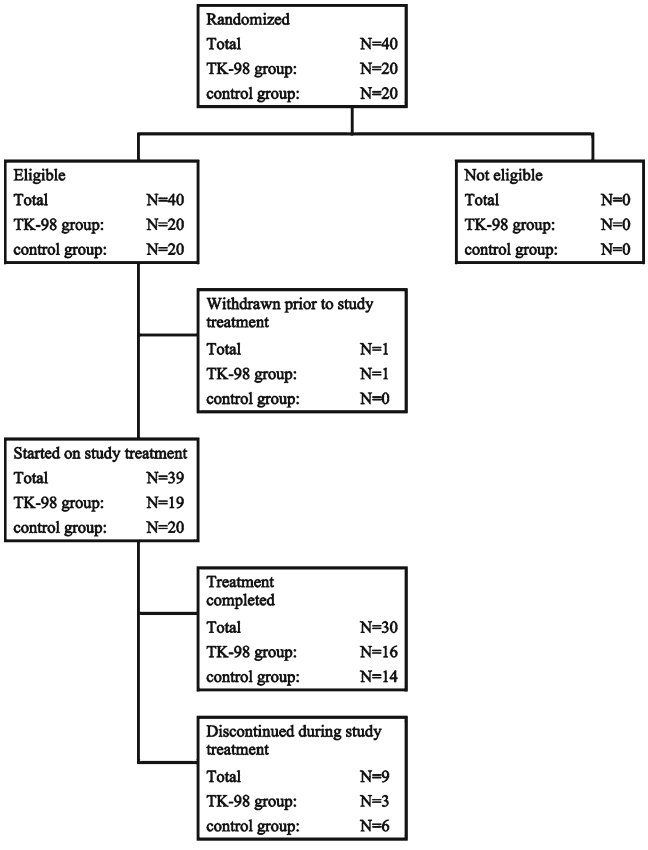

Fig. 1. Flow chart of patients. A total of 39 subjects who initiated study treatment were included in the full analysis set (FAS) and the per protocol set (PPS), as well as a safety analysis

One patient in the control group was positive for anti-hepatitis B surface antigen, but negative for anti-hepatitis B e antigen and with a low anti-hepatitis B core (HBc) antibody titer. The patient's serum hepatitis B virus (HBV) DNA level remained at <2.6 log copies/ml; therefore, the hepatic disorder in this patient was considered to be due mainly to HCV. All patients were negative for antinuclear antibodies and antimitochondrial-M2 antibodies, indicating no concurrent autoimmune hepatitis or primary biliary cirrhosis. A positive anti-HBc antibody result was reported in 12 patients (63.2%) in the TK-98 group (n = 19) and in 11 patients (55.0%) in the control group (n = 20). Of these patients, HCC developed in three patients in each group. High serum anti-HBc antibody titers were observed in four patients (21.1%) of the TK-98 group four (20.0%) of the control group, among whom only one patient of the TK-98 group contracted HCC.

Ursodeoxycholic acid (UDCA) was used in 13 patients (68.4%) in the TK-98 group and in 17 patients (85.0%) in the control group, and parenteral glycyrrhizinate was administered to 14 patients (73.7%) of the TK-98 group and 12 patients (60.0%) of the control group. Of the eight patients with HCC, seven received both UDCA and parenteral glycyrrhizinate. Interferon was used in one patient (5.0%) of the control group.

Primary end point

During the 168-week observation period, no patients had symptoms of ascites, edema, or hepatic encephalopathy indicating decompensated cirrhosis in either the TK-98 group or the control group. Therefore, analysis for primary end-point assessment was not performed.

Secondary variables

No remarkable findings were noted regarding jaundice or PS in the two groups. The time courses of the serum albumin level and Fischer's ratio are presented in Figs. 2 and 3, respectively. The serum albumin levels (mean ± SD) at baseline and at weeks 56, 112, and 168 of study observation were 3.86 ± 0.26, 3.82 ± 0.24, 3.81 ± 0.19, and 3.73 ± 0.29, respectively, in the TK-98 group, and 3.90 ± 0.33, 3.91 ± 0.29, 3.91 ± 0.28, and 4.03 ± 0.30, respectively, in the control group (Table 2). A group-effect analysis of the serum albumin levels revealed no significant differences between the two groups (P = 0.8488). A mixed effect model was used to analyze changes in serum albumin levels over time during the 168-week period, using the study group and the assessment time point as

Table 1. Baseline characteristics of two groups

	TK-98 group (n = 19)	Control group (n = 20)
Age (years)	62.9 ± 5.7	59.5 ± 7.2
Height (cm)	165.07 ± 6.46	166.94 ± 4.48
Body weight (kg)	62.81 ± 9.41	68.39 ± 10.64
BMI (kg/m^2)	23.03 ± 3.03	24.51 ± 3.50
Time since contraction of disease (years)	4.86 ± 4.64	4.29 ± 3.86
History of alcohol consumption (yes/no)	6/13	6/14
Ascites	0	0
Edema	0	0
Hepatic encephalopathy	0	0
Gastric and esophagus varices	10	10
Concurrent of diabetes mellitus	3	4
Concurrent hypertension	7	6
Concurrent gallstone	4	3
Platelet count (×10^4/mm^3)	12.23 ± 6.48	11.59 ± 4.33
Total protein (g/dl)	7.73 ± 0.47	7.64 ± 0.37
Serum albumin (g/dl)	3.86 ± 0.26	3.90 ± 0.33
Total bilirubin (mg/dl)	0.77 ± 0.23	0.75 ± 0.22
AFP (mAU/ml)	11.0 ± 12.9	10.9 ± 10.9
PIVKA-II (ng/ml)	21.5 ± 11.6	19.5 ± 7.1
Fischer's ratio	3.047 ± 0.637	2.734 ± 0.647
AST (GOT) (IU/l)	42.9 ± 16.3	41.8 ± 14.6
ALT (GPT) (IU/l)	48.0 ± 24.2	47.7 ± 23.3
HBsAg (+)	0	1
HBcAb (+)	12	11
HBcAb (+) (high titer)[a]	4	4
ANA (+)	0	0
AMA-M2 (+)	0	0

Data are expressed as number of patients or mean ± standard deviation
BMI, body mass index; AFP, a-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist II; AST, aspartate aminotransferase; GOT, glutamyl oxaloacetic transaminase; ALT, alanine aminotransferase; GPT, glutamyl pyruvic transaminase; HBsAg, hepatitis B surface antigen; HBcAb, hepatitis B core antibody; ANA, antinuclear antibody; AMA-M2, anti-mitochondrial antibody-M2; S/CO, sample/cut off
[a] S/CO score ≥ 10.00 (CLIA method)

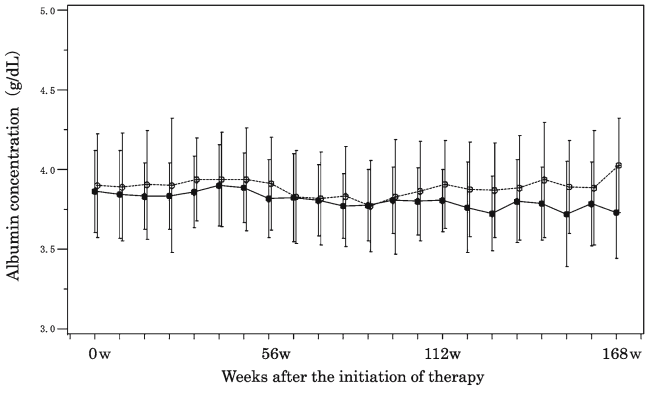

Fig. 2. Serum albumin concentration in TK-98 group (*black dots*) and control group (*white dots*). Data are means; bars show the standard deviation

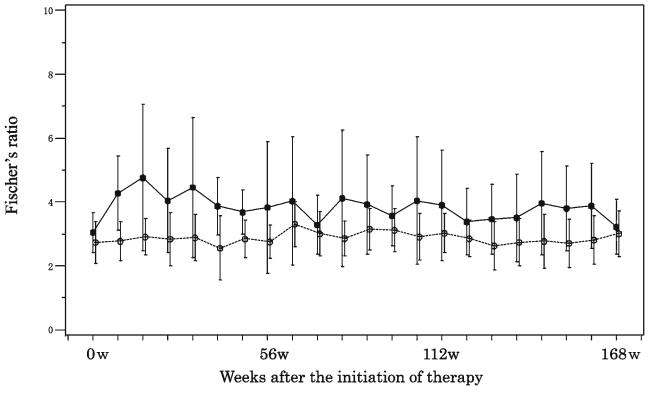

Fig. 3. Fischer's ratio in TK-98 group (*black dots*) and control group (*white dots*). Data are means; bars show the standard deviation

interaction terms, and resulted in an estimate of –0.005, P = 0.0288. These findings implied that the intergroup difference in serum albumin levels widened progressively by –0.005 g/dl every 8 weeks. However, these changes were negligible with respect to the time course of serum albumin levels over the 168 weeks.

A group-effect analysis revealed that Fischer's ratio was significantly higher in TK-98 treated patients (P =

Table 2. Mixed-effects model analysis of the pattern of changes in serum albumin levels and Fischer's ratio

	Baseline	Week 56	Week 112	Week 168	Group effect			Time point × group interaction		
Group	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Estimate	*t*	*P*	Estimate	*t*	*P*
Serum albumin levels										
TK-98 group	3.86 ± 0.26	3.82 ± 0.24	3.81 ± 0.19	3.73 ± 0.29	0.01157	0.19	0.8488	–0.00497	–2.19	0.0288
Control group	3.90 ± 0.33	3.91 ± 0.29	3.91 ± 0.28	4.03 ± 0.30						
Fischer's ratio										
TK-98 group	3.05 ± 0.64	3.83 ± 2.06	3.91 ± 1.74	3.22 ± 0.86	0.3054	4.10	0.0001	–0.00883	–2.46	0.0143
Control group	2.73 ± 0.65	2.75 ± 0.52	3.02 ± 0.61	3.01 ± 0.72						

Table 3. Cox proportional hazards model analysis of the event of hepatocellular carcinoma

	Hazard ratio	95% confidence interval	χ^2	Two-sided *P* value
Analysis with treatment group as an independent variable				
Independent variable				
Treatment group	0.606	0.145–2.539	0.4690	0.4935
Analysis with treatment group as an independent variable and serum albumin level as a explanatory variable				
Independent variable				
Treatment group	0.546	0.130–2.299	0.6808	0.4093
Explanatory variable				
Albumin	0.452	0.058–3.522	0.5755	0.4481

0.0001). Fischer's ratio (mean ± SD) at baseline and at weeks 56, 112 and 168 of study observation was 3.047 ± 0.637, 3.831 ± 2.056, 3.905 ± 1.735, and 3.221 ± 0.862, respectively, in the TK-98 group, and 2.734 ± 0.647, 2.754 ± 0.521, 3.021 ± 0.614, and 3.012 ± 0.715 in the control group (Table 2).

HCC developed in three of 19 patients in the TK-98 group and in five of 20 in the control group. Cox's proportional hazards model analyses were performed to determine the effect of BCAA treatment and serum albumin levels on development of HCC. The results showed that the hazard ratio of the BCAA treatment relative to no treatment was 0.606 (95% confidence interval, 0.145–2.539; Table 3). A time-to-event analysis was performed with the development of HCC. The result was $P = 0.4889$ (log-rank test, Fig. 4). Furthermore, another time-to-event analysis for subgroups with baseline body mass index (BMI) of 25 and higher or those with a baseline serum albumin level of ≤4.0 g/dl yielded $P = 0.2473$ and $P = 0.0930$ (log-rank test), respectively, in these two subgroups (Fig. 5).

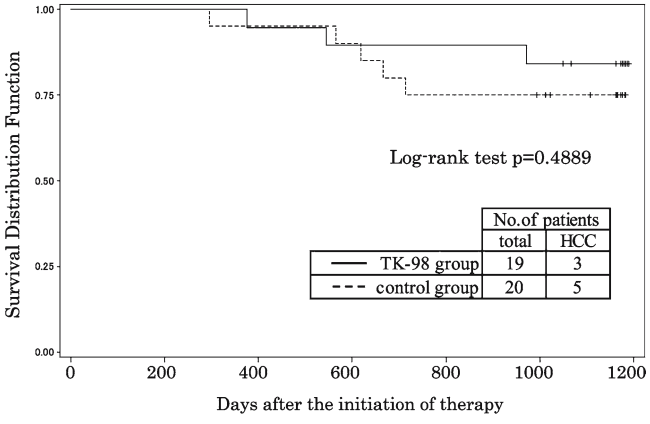

Fig. 4. Kaplan-Meier estimates of event-free survival for hepatocellular carcinoma (*HCC*) in patients with compensated liver cirrhosis caused by hepatitis C virus (HCV) infection

Safety

During the study, adverse events were reported in 17 (89.5%) of 19 patients treated with TK-98 (75 events) and in 19 (95.0%) of 20 untreated patients (85 events). No significant difference was found in the incidence of adverse events between the two groups. Two adverse reactions were reported in TK-98 treated patients: constrictive pericarditis in one patient, and a gastrointestinal symptom in another.

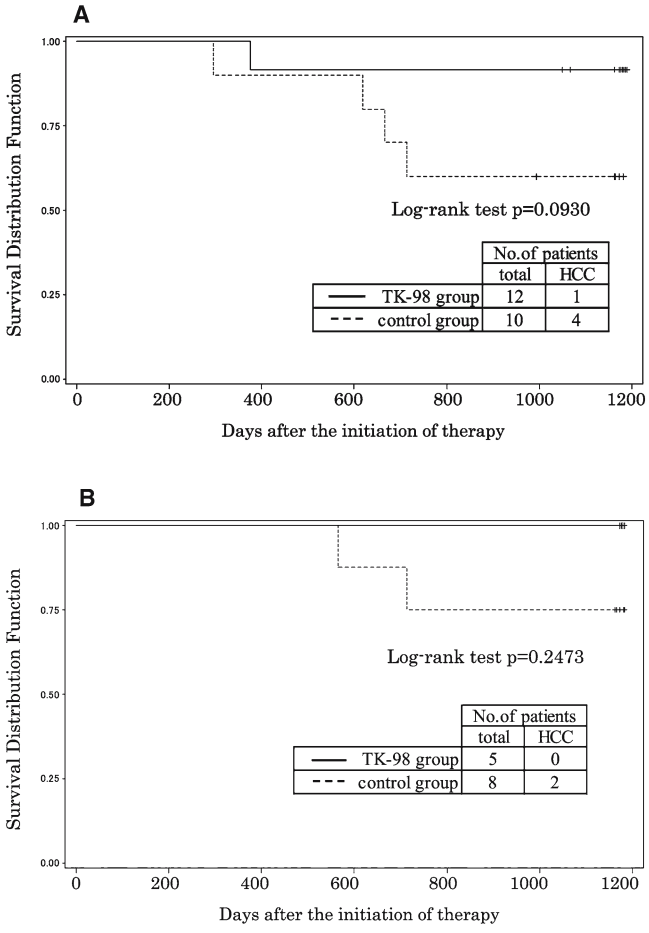

Fig. 5A,B. Kaplan-Meier estimates of event-free survival for HCC in patients with compensated liver cirrhosis caused by HCV infection. **A** Subgroup with a baseline serum albumin level of <4.0 g/dl; **B** subgroup with baseline body mass index (*BMI*) ≥ 25

Discussion

In Japan, no effective treatment has been established for compensated cirrhosis, whereas an effect of BCAA therapy has been confirmed in patients with decompensated cirrhosis and a serum albumin level of <3.5 g/dl. Several studies have shown an effect of BCAA in patients with compensated cirrhosis by investigating influence of the therapy on serum albumin levels,[11,12] but no studies have been performed to investigate the effect of BCAA on the entire disease state of liver cirrhosis. Therefore, we conducted a randomized controlled trial on the presumption that treatment with BCAA in patients with compensated cirrhosis might possibly delay disease progression.

In the present study, we assumed that the disease phase might shift to decompensated cirrhosis in several of the patients randomized to the control group. In the course of the 168-week observation period, however, no appreciable changes in serum albumin levels or Fischer's ratio were found in this group. Also, no symptoms of ascites, edema, or hepatic encephalopathy, indicating decompensated cirrhosis, developed. The results therefore failed to demonstrate any inhibitory effect of BCAA on progression from compensated to decompensated cirrhosis. A slightly extended observation period and a larger sample size would be necessary to identify such an effect of BCAA.

The mechanism whereby BCAA can improve hypoalbuminemia has been considered to consist in the supply of substrates for protein synthesis from a nutritional standpoint. Later, it was clarified that BCAA, especially L-leucine, acts to facilitate protein synthesis by stimulating initiation of albumin mRNA translation via activation of the intracellular signal transduction system, primarily pertaining to mammalian target of rapamycin (mTOR).[13,14] A study assessing albumin synthesis in primary cultures of rat hepatocytes with BCAA showed that the albumin level increased in the presence of BCAA from 0.1 to 0.5 mM in a dose-dependent fashion, whereas there was no such elevation in the albumin concentration at higher levels of BCAA.[14]

Habu and colleagues reported the effect of BCAA on serum albumin levels in relation to the BCAA/tyrosine molar ratio (BTR) in studies in which they administered BCAA granules for 2 years to patients with compensated cirrhosis with serum albumin levels between 3.5 and 3.9 g/dl. They showed that the BCAA treatment increased serum albumin levels in patients with cirrhosis and with BTR < 4, whereas there was no appreciable elevation in serum albumin levels in patients with BTR ≥ 4.[11,12] The BTR has been reported to correlate well with Fischer's ratio,[15] and a BTR value of 4 corresponds to a Fischer's ratio of 2.[11] In the present study, nearly all patients had a baseline Fischer's ratio of 2 or greater, and the BTR value was maintained without any decrease during the study. Our results revealed that an albumin-increasing effect of BCAA treatment was unclear in patients with compensated cirrhosis and a Fischer's ratio of 2 or higher, which is consistent with the findings of Habu and colleagues. We thus inferred that no appreciable elevation in serum albumin level occurs in response to treatment with BCAA of patients with cirrhosis but without an amino acid imbalance.

HCC developed in three (15.8%) of the 19 TK-98 treated patients and in five (25.0%) of the 20 untreated patients (control). There was no evidence of an inhibitory effect of BCAA treatment on the development of HCC (Fig. 4). A previous study indicated that, in patients with cirrhosis due to HCV infection, the lower the serum albumin level, the greater the risk for hepatic carcinogenesis, and that the hazard ratio in this respect was 1.92-fold higher in patients with cirrhosis and a serum albumin level of <4.0 g/dl than in those with a serum albumin level of 4.0 g/dl or higher.[6] Another study dem-

onstrated that BCAA suppressed cancer development in patients with decompensated cirrhosis and a BMI of ≥25.[16] In the present study, we also performed a time-to-event analysis of pertinent data from a subset of patients with BMI ≥ 25 or those with a baseline serum albumin level of <4.0 g/dl to explore for any suppressive effect of BCAA on hepatic carcinogenesis, using the development of HCC as the event. The analysis revealed a tendency toward suppression of hepatic cancer development in the subgroup with a baseline serum albumin level of <4.0 g/dl ($P = 0.0930$, log-rank test), but the P value was 0.2473 (log-rank test) for the subgroup with BMI ≥ 25 (Fig. 5).

It is generally recognized that abnormal carbohydrate metabolism occurs frequently in patients with cirrhosis due to HCV infection,[17] and the incidence is higher in patients presenting with more advanced symptoms. Hyperinsulinemia and insulin resistance have been identified as major factors contributing to the development of abnormal carbohydrate metabolism, and recent studies have implicated hyperinsulinemia and obesity as risk factors in the genesis of HCC.[18–22] Furthermore, another study has documented acceleration of HCC proliferation in the presence of postprandial hyperinsulinemia.[23]

Recent studies using a CCl_4-induced rat cirrhosis model have demonstrated that L-leucine and L-isoleucine improve abnormal carbohydrate metabolism by facilitating non-insulin-mediated glucose uptake in skeletal muscles and by stimulating m-TOR signaling-mediated glycogen synthesis.[24–26] We thus infer that in patients with cirrhosis and abnormal glucose tolerance, BCAA treatment provides correction of hyperinsulinemia via improvement of abnormal carbohydrate metabolism. Therefore, our results showing that hepatic cancer development tended to be suppressed following treatment with BCAA may indicate an effect of BCAA in ameliorating abnormal carbohydrate metabolism. In fact, the large-scale LOTUS study conducted in patients with decompensated cirrhosis demonstrated that long-term dietary supplementation with BCAA inhibited liver carcinogenesis in patients with cirrhosis and BMI ≥ 25, who are often considered to have hyperinsulinemia or insulin resistance.[16] However, blood glucose and insulin were not determined in this study, so assessment of the effect of BCAA on carbohydrate metabolism is left for future studies.

The present study, though of a small scale, represents the first clinical trial ever undertaken to explore the inhibitory effect of BCAA on disease progression in patients with compensated cirrhosis. No symptoms indicating progression of cirrhosis from the compensated to decompensated phase were noted in either the TK-98 group or the control group during this study, and we could not evaluate any inhibitory effect of BCAA therapy on progression of cirrhosis. However, the results suggested that BCAA may inhibit hepatic carcinogenesis in patients with compensated cirrhosis and a serum albumin level of <4 g/dl. Long-term therapy with BCAA granules is not considered to entail any safety concerns because there was no statistically significant difference between the two groups in the incidence of adverse events, nor was there any adverse event of clinical concern.

BCAA has a variety of pharmacologic effects, among which the effect of improving abnormal carbohydrate metabolism is considered to have an inhibitory effect on liver carcinogenesis. The underlying mechanism of this action, nevertheless, has yet to be further clarified. It is important to explore whether BCAA therapy inhibits development of hepatic or other types of cancer in larger clinical trials with patients with compensated cirrhosis.

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