Clinical significance of early interventional therapy of branched-chain amino acid granules in patients with hepatocellular carcinoma: Propensity score matching analysis

HIROKI NISHIKAWA, RYUICHI KITA, TORU KIMURA, YOSHIAKI OHARA, HARUHIKO TAKEDA, AZUSA SAKAMOTO, SUMIO SAITO, NORIHIRO NISHIJIMA, AKIHIRO NASU, HIDEYUKI KOMEKADO and YUKIO OSAKI

Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Osaka 543-0027, Japan

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Abstract. We examined whether supplementation of branched-chain amino acid (BCAA) granules in an early stage of underlying liver disease (pretreatment serum albumin levels ≥3.6 g/dl) can improve overall survival (OS) after therapy for hepatocellular carcinoma (HCC) using propensity score matching analysis. We compared OS between patients treated with BCAA granules and control group patients in two propensity score matched cohorts (cohort 1: pretreatment serum albumin levels ≥3.6 g/dl and <4.0 g/dl, 111 pairs; cohort 2: pretreatment serum albumin levels ≥4.0 g/dl, 61 pairs). We also performed subgroup analyses according to HCC stage. In cohort 1 patients, the OS rate in the BCAA group (median follow-up period, 2.9 years) tended to be higher compared to that in the control group (median follow-up period, 2.6 years) (1- and 3-year OS rates; 97.2 and 75.5% in the BCAA group and 87.2 and 64.5% in the control group, P=0.072), whereas in cohort 2 patients, the difference in the two groups did not reach significance in terms of OS [1- and 3-year OS rates; 83.2 and 60.7% in the BCAA group (median follow-up period, 2.3 years) and 91.8 and 66.0% in the control group (median follow-up period, 2.9 years), P=0.871]. In subgroup analyses, in cohort 1, in patients with HCC stage III or IV, the OS rate in the BCAA group (n=37) was significantly higher compared to that in the control group (n=34) (P=0.017). In other subgroup analyses, no significant difference in the two groups was found in terms of OS. In conclusion, early interventional therapies using BCAA granules may be effective in some selected HCC patients.

Correspondence to: Dr Hiroki Nishikawa, Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, 5-30 Fudegasaki-cho, Tennoji-ku, Osaka 543-0027, Japan
E-mail: h-nishikawa@osaka-med.jrc.or.jp

Key words: hepatocellular carcinoma, branched-chain amino acid granules, early intervention, survival, propensity score matching

Introduction

Hepatocellular carcinoma (HCC) is the most common carcinoma worldwide (1). Unlike most solid cancers, both the incidence and mortality rate for HCC patients are expected to increase substantially in many countries over the next 20 years, mostly as a result of infection with hepatitis C virus (HCV), in Japan, however, the incidence of non-B and non-C HCC has recently tended to increase (2,3). Treatment methods for HCC vary depending on the disease stage and liver function, and they include surgical resection (SR), liver transplantation, radiofrequency thermal ablation (RFA), percutaneous ethanol injection therapy, transcatheter arterial chemotherapy with or without embolization, systemic treatment with molecular-targeted therapy (MTT) such as sorafenib therapy and radiation therapy (RT) (4-7). HCC also carries a considerable risk of tumor recurrence even when curative treatment was performed at the initial therapy, with the tumor characteristics and any underlying liver disease important predictive factors affecting the risk of HCC recurrence (1,2,4,6).

Branched-chain amino acids (BCAAs) are a group of essential amino acids comprising valine, leucine, and isoleucine. A low plasma level ratio of BCAAs to aromatic amino acids suggests liver cirrhosis (LC) physiologically, and BCAA supplementation was originally developed in order to normalize the patient's amino acid profile and nutritional status (8-11). Most HCC patients have underlying various stages of LC including compensated or decompensated stages. LC patients with decreased plasma BCAA level can develop protein-energy malnutrition (PEM) with increased catabolism (12). PEM is associated with a high morbidity and mortality due to an increased risk of life-threatening complications, resulting in poor clinical outcome and deteriorated quality of life (QOL) (9). PEM in LC patients is already observed in the compensated phase with serum albumin level, which is a useful indicator of liver functional reserve, ≥3.6 g/dl (13).

Supplementation with BCAA for the treatment of patients with liver disorder has been attracting attention. BCAA has a variety of pharmacological effects. BCAA treatment can correct malnutrition associated with LC (14,15) and long-term nutritional BCAA supplementation may be effective for increasing plasma BCAA levels, albumin synthesis and prevention of hepatic failure while it also improves surrogate markers in patients with advanced LC (16,17). On the other hand, dietary supplementation alone does not affect plasma BCAA levels in patients with LC (18).

BCAA granules (Livact; Ajinomoto Pharmaceutical, Tokyo, Japan) have been approved for its use in LC patients since 1996 in Japan, and BCAA granules are generally administered in LC patients with a serum albumin level ≤3.5 g/dl. Clinical evidence regarding the effect of this therapy is being accumulated (19-23). Thus, the 2008 Japanese guidelines for the treatment of patients with chronic liver diseases recommend that treatment with BCAA granules should be performed for decompensated LC with a serum albumin level ≤3.5 g/dl (19). In Japan, BCAA granules are widely used in clinical practice without serious adverse effects. In addition, we have previously demonstrated that BCAA treatment may improve OS and recurrence-free survival after RFA in patients with HCV-related HCC ≤3 cm in diameter with ≤3 nodules and a serum albumin level before RFA ≤3.5 g/dl (24). On the other hand, several investigators reported that for patients with chronic liver diseases including LC, it might be beneficial to initiate BCAA therapy in the compensatory stage or even earlier, which means 'early intervention using BCAA therapy', rather than starting BCAA therapy in the decompensatory stage (25-27). Thus, BCAA supplementation may be effective in improving clinical outcome in cirrhotic patients regardless of disease stage.

However, to our knowledge, whether this early intervention using BCAA granules in patients with HCC can contribute to prolongation of survival remains unclear. The objectives of the present study were to examine whether supplementation of BCAA granules in an early stage of underlying liver disease (pretreatment serum albumin level ≥3.6 g/dl) can improve overall survival (OS) after HCC therapy. For reducing selection biases, we used propensity score matching analysis.

Patients and methods

Patients. A total of 1,134 consecutive treatment-naïve patients diagnosed with HCC with pretreatment serum albumin level ≥3.6 g/dl were admitted to the Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Japan, between 2004 and 2013. We divided these patients into two groups according to pretreatment serum albumin level (cut-off serum albumin level, 4.0 g/dl). There were 470 patients with pretreatment serum albumin level ≥3.6 g/dl and <4.0 g/dl at the initial treatment for HCC. Of these, BCAA granules were prescribed in 120 patients at the initial therapy for HCC and the remaining 350 patients did not receive such therapy at the initial therapy for HCC. Since this study was a retrospective observational study, covariate adjustment using the propensity score was performed. One hundred and eleven pairs were thus selected for analysis in this cohort (cohort 1) (Fig. 1). Similarly, there were 664 patients with pretreatment serum albumin level ≥4.0 g/dl at the initial treatment for HCC. Of these, BCAA granules were prescribed in 63 patients at the initial therapy for HCC and the remaining 601 patients did not receive such therapy at the initial therapy for HCC. After using the propensity score matching, 61 pairs were selected for analysis in this cohort (cohort 2) (Fig. 1). We compared the OS rate between the BCAA group and the control group in each cohort.

Prior to therapy for HCC, written informed consent was obtained from all patients. The ethics committee of our department approved the protocol for HCC therapy. The present study comprised a retrospective analysis of patients' medical records in our database and all treatments were performed in an open-label manner.

Diagnosis of HCC and HCC therapy. HCC was diagnosed based on the results from abdominal ultrasound and dynamic computed tomography (CT) scan (hyperattenuation during the arterial phase in the entire or part of the tumor, and hypoattenuation in the portal-venous phase) and/or magnetic resonance imaging mainly as recommended by the American Association for the Study of Liver Diseases (28). Arterial and portal phase dynamic CT images were obtained ~30 and 120 sec after injection of contrast material. For all patients, abdominal angiography combined with CT (angio-CT) was performed before therapy for HCC after obtaining informed consent from them for performing abdominal angiography. This was performed based on the fact that this technique was useful for detecting small satellite nodules as reported by Yamasaki *et al* (29). Then, we confirmed HCC using CT during hepatic arteriography (CTHA) and CT during arterial-portography (CTAP). As for HCC therapy, the most appropriate treatment modality for each patient was selected through discussion with surgeons, hepatologists and radiologists (30,31). In the present analysis, there was no patient treated with liver transplantation and there was no treatment related death.

BCAA granule treatment. The patient's attending physician determined whether treatment with BCAA granules would be performed in individual patients considering their intent to receive the treatment after providing sufficient information regarding BCAA treatment to them. In the BCAA group, BCAA granules, containing 952 mg of L-isoleucine, 1,904 mg of L-leucine and 1,144 mg of L-valine per sachet, were orally administered to subjects at a dose of one sachet three times daily after meals ≥1 month after initial therapy for HCC. We confirmed in our database that patients in the BCAA group were prescribed BCAA granules regularly.

Follow-up after initial therapy for HCC. Follow-up observation consisted of regular blood tests and monitoring of tumor markers, including α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), which was measured using a chemiluminescent enzyme immunoassay (Lumipulse PIVKAII Eisai, Eisai, Tokyo, Japan). Dynamic CT scan was performed every 3-4 months after initial therapy for HCC. In particular, for patients in the BCAA group, we confirmed that BCAA granules were taken properly at every hospital visit. When HCC recurrence or disease progression was detected based on radiologic findings, most appropriate therapy was performed in each patient.

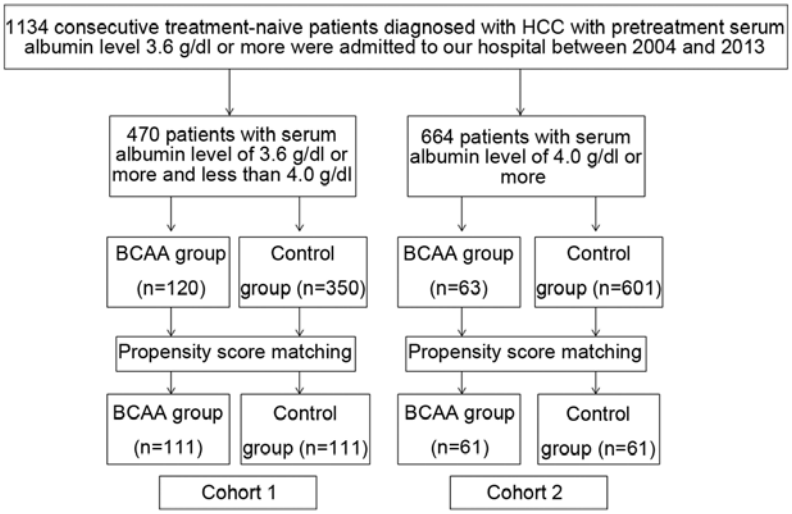

Figure 1. Study design.

Statistical analysis. The primary end-point is OS. Continuous variables were compared by unpaired t-test, and categorical variables were compared by Fisher's exact test. Data were analyzed using univariate and multivariate analyses. The cumulative OS rate was calculated by Kaplan-Meier method and tested by log-rank test. A Cox proportional hazard model was used for multivariate analyses of factors with P<0.1 in univariate analysis. These statistical methods were used to estimate the interval from each initial therapy for HCC. Data were analyzed using SPSS software (SPSS Inc., Chicago, IL, USA) for Microsoft Windows. Data are expressed as mean ± standard deviation. A P-value <0.05 was considered to be statistically significant.

Propensity score analysis. To compare the OS between BCAA group patients and control group patients, a propensity score model was used with an attempt to reduce potential biases in survival analysis (32,33). Possible variables associated with long-term survival of HCC patients, including age, sex, HCC stage, maximum tumor size, cause of liver disease, serum albumin level, aspartate aminotransferase (AST) value and alanine aminotransferase (ALT) value were included comprehensively for propensity score generation. With these selected variables, a logistic regression was applied to generate a continuous propensity score from 0 to 1. One-to-one matches between BCAA group patients and control group patients were introduced into the subsequent analysis.

Results

Patient demographic characteristics and survival (cohort 1). Baseline demographic characteristics of patients in cohort 1 are shown in Table I. There were 111 patients in the BCAA group and 111 patients in the control group, respectively. In terms of baseline demographic characteristics, no significant differences were noted between the BCAA group and the control group, showing that balance of baseline characteristics in the two groups was obtained in the matched sample. As an initial therapy for HCC, in the BCAA group, SR was performed in 35 patients, percutaneous ablative therapies in 52, transcatheter arterial chemotherapy with or without embolization in 22, MTT in one and RT in one, whereas in the control group, SR was performed in 23 patients, percutaneous ablative therapies in 64, transcatheter arterial chemotherapy with or without embolization in 21 and MTT in three (P=0.201).

The median follow-up period was 2.9 years (range, 0.5-7.0 years) in the BCAA group and 2.6 years (range, 0.1-7.6 years) in the control group. Thirty-nine patients (35.2%) in the BCAA group died during the follow-up period. The causes of death were HCC progression (24 patients), liver failure (12 patients) and miscellaneous (3 patients). Sixty patients (54.1%) in the control group died during the follow-up period. The causes of death were HCC progression (40 patients), liver failure (14 patients) and miscellaneous (6 patients).

The 1-, 3- and 5-year OS rates after each initial therapy for HCC were 97.2, 75.5 and 56.3%, respectively, in the BCAA group and 87.2, 64.5 and 38.8%, respectively, in the control group (P=0.072) (Fig. 2), indicating that the OS rate in the BCAA group tended to be higher compared to that in the control group.

Univariate and multivariate analysis of factors contributing to OS (cohort 1). In patients with pretreatment serum albumin level of ≥3.6 and <4.0 g/dl, using univariate analyses of factors contributing to OS, HCC stage (P<0.001), maximum tumor size ≥2.5 cm (P=0.004), AST ≥50 IU/l (P=0.004), ALT ≥40 IU/l (P=0.018), alkaline phosphatase (ALP) ≥330 IU/l (P=0.013), γ glutamyl transpeptidase (GGT) ≥70 IU/l (P=0.005), AFP ≥100 ng/ml (P=0.001) and DCP ≥100 mAU/ml (P<0.001) were found to be significant factors (Table II). The multivariate analyses involving nine factors with P<0.1 in the univariate analysis showed that only HCC stage was a significant independent predictor linked to OS (P=0.001). The hazard ratios (HRs), 95% confidence interval (CI) and P-value for nine factors are detailed in Table II.

Patient demographic characteristics and survival (cohort 2). Baseline demographic characteristics of patients in cohort 2 are shown in Table III. They included 61 patients in the BCAA group and 61 patients in the control group, respectively. In

Table I. Baseline characteristics between the BCAA group and the control group in HCC patients with pretreatment serum albumin level ≥3.6g/dl and <4.0 g/dl after propensity score matching.

Variables	BCAA group (n=111)	Control group (n=111)	P-value
Age (years)	67.8±9.3	69.8±9.6	0.144[a]
Gender, male/female	77/34	75/36	0.885[b]
Causes of liver disease			
B/C/non-B non-C/B and C	7/71/32/1	9/79/23/0	0.348[b]
HCC stage I/II/III/IV	27/47/31/6	26/51/23/11	0.417[b]
Maximum tumor size (cm)	3.2±2.3	3.4±2.7	0.665[a]
Initial therapy for HCC			
SR/ablation/transcatheter arterial chemotherapy/MTT/RT	35/52/22/1/1	23/64/21/3/0	0.201[b]
AST (IU/l)	57.1±28.3	63.8±41.6	0.170[a]
ALT (IU/l)	48.5±31.5	56.4±51.2	0.164[a]
ALP (IU/l)	383.8±165.3	406.5±331.5	0.520[a]
GGT (IU/l)	96.9±86.8	119.3±152.5	0.158[a]
Serum albumin (g/dl)	3.8±0.1	3.7±0.1	0.684[a]
Total bilirubin (mg/dl)	1.0±0.5	1.0±0.6	0.419[a]
Prothrombin time (%)	81.8±12.9	84.1±13.9	0.180[a]
Platelets ($x10^4/mm^3$)	10.8±5.8	11.4±4.1	0.194[a]
AFP (ng/ml)	1,548±12,111	2,918±25,034	0.604[a]
DCP (mAU/ml)[c]	7,570±49,443	3,372±12,614	0.387[a]

Data are expressed as number or mean ± standard deviation. BCAA, branched-chain amino acid; HCC, hepatocellular carcinoma; MTT, molecular targeting therapy; RT, radiation therapy; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin. [a]Unpaired t-test; [b]Fisher's exact test; [c]missing data, n=1.

Table II. Univariate and multivariate analysis of factors contributing to overall survival in HCC patients with pretreatment serum albumin level ≥3.6 and <4.0 g/dl (cohort 1).

Variables	n	Univariate analysis P-value[a]	Multivariate analysis Hazard ratio (95% CI)	Multivariate analysis P-value[b]
Gender, male vs. female	152/70	0.558		
Age (years), ≥70 vs. <70	111/111	0.739		
BCAA vs. control	111/111	0.072	1.372 (0.887-2.123)	0.155
HCC stage I or II vs. III or IV	151/71	<0.001	0.376 (0.214-0.659)	0.001
Maximum tumor size (cm), ≥2.5 vs. <2.5	105/117	0.004	0.964 (0.575-1.616)	0.889
Cause of liver disease, viral vs. non-viral	167/55	0.523		
AST (IU/l), ≥50 vs. <50	116/106	0.004	0.731 (0.387-1.380)	0.333
ALT (IU/l), ≥40 vs. <40	111/111	0.018	0.962 (0.520-1.780)	0.903
ALP (IU/l), ≥330 vs. <330	116/106	0.013	0.804 (0.515-1.254)	0.336
GGT (IU/l), ≥70 vs. <70	111/111	0.005	0.884 (0.563-1.389)	0.593
Serum albumin level (g/dl), ≥3.8 vs. <3.8	108/114	0.374		
Total bilirubin (mg/dl), ≥1.0 vs. <1.0	90/132	0.117		
Platelet count ($x10^4/mm^3$), ≥10 vs. <10	106/116	0.472		
Prothrombin time (%), ≥70 vs. <70	187/35	0.687		
Serum AFP (ng/ml), ≥100 vs. <100	62/160	0.001	0.788 (0.500-1.242)	0.305
DCP (mAU/ml), ≥100 vs. <100[c]	78/143	<0.001	0.767 (0.458-1.282)	0.311

BCAA, branched-chain amino acid; HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; CI, confidence interval. [a]Log-rank test; [b]Cox proportional hazard model; [c]missing data, n=1.

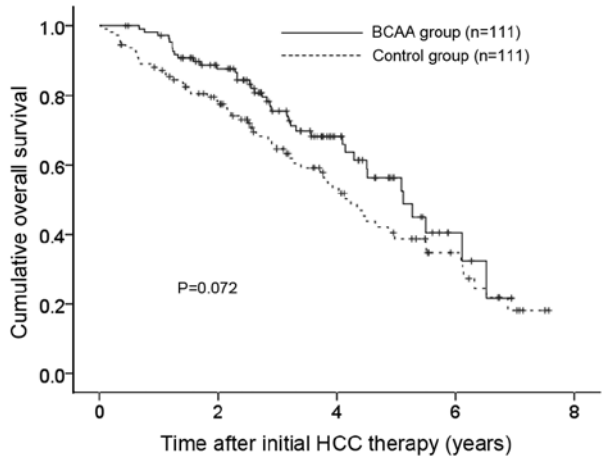

Figure 2. Cumulative overall survival in patients with pretreatment serum albumin level ≥3.6 and <4.0 g/dl (cohort 1). The 1-, 3- and 5-year OS rates after each therapy for HCC were 97.2, 75.5 and 56.3%, respectively, in the BCAA group (n=111) and 87.2, 64.5 and 38.8%, respectively, in the control group (n=111) (P=0.072).

terms of baseline demographic characteristics, no significant differences were noted between the BCAA group and the control group, demonstrating that balance of baseline characteristics in the two groups was obtained in the matched sample. As an initial therapy for HCC, in the BCAA group, SR was performed in 31 patients, percutaneous ablative therapies in 15 and transcatheter arterial chemotherapy with or without embolization in 15, whereas in the control group, SR was performed in 20 patients, percutaneous ablative therapies in 19, transcatheter arterial chemotherapy with or without embolization in 21 and MTT in one (P=0.160).

The median follow-up period was 2.3 years (range, 0.2-7.8 years) in the BCAA group and 2.9 years (range, 0.3-8.4 years) in the control group. Twenty-four patients (39.3%) in the BCAA group died during the follow-up period. The causes of death were HCC progression (22 patients) and liver failure (2 patients). Twenty-eight patients (45.9%) in the control group died during the follow-up period. The causes of death were HCC progression (24 patients), liver failure (2 patients) and miscellaneous (2 patients).

The 1-, 3- and 5-year OS rates after each initial therapy for HCC were 83.2, 60.7 and 54.0%, respectively, in the BCAA group and 91.8, 66.0 and 44.5%, respectively, in the control group (P=0.871) (Fig. 3).

Univariate and multivariate analysis of factors contributing to OS (cohort 2). In patients with serum albumin level of ≥4.0 g/dl, using univariate analyses of factors contributing to OS, HCC stage (P<0.001), maximum tumor size ≥4.0 cm (P<0.001), AST ≥50 IU/l (P=0.024), ALP ≥300 IU/l (P<0.001), GGT ≥70 IU/l (P=0.009), total bilirubin ≥1.0 mg/dl (P=0.045), AFP ≥100 ng/ml (P=0.013) and DCP ≥100 mAU/ml (P<0.001) were found

Table III. Baseline characteristics between the BCAA group and the control group in HCC patients with pretreatment serum albumin level ≥4.0 g/dl after propensity score matching.

Variables	BCAA group (n=61)	Control group (n=61)	P-value
Age (years)	69.8±10.0	69.9±9.7	0.985[a]
Gender, male/female	43/18	42/19	>0.999[b]
Causes of liver disease			
B/C/non-B non-C/B and C	10/29/21/1	8/31/21/1	0.954[b]
HCC stage I/II/III/IV	6/26/20/9	7/27/23/4	0.555[b]
Maximum tumor size (cm)	4.9±2.9	4.7±3.3	0.686[a]
Initial therapy for HCC			
SR/ablation/transcatheter arterial chemotherapy/MMT	31/15/15/0	20/19/21/1	0.160[b]
AST (IU/l)	67.0±54.7	59.3±36.4	0.183[a]
ALT (IU/l)	55.0±37.2	54.1±47.4	0.905[a]
ALP (IU/l)	365.5±204.7	320.6±131.5	0.139[a]
GGT (IU/l)	128.9±151.7	133.0±145.3	0.879[a]
Serum albumin (g/dl)	4.2±0.2	4.3±0.2	0.344[a]
Total bilirubin (mg/dl)	1.0±0.4	0.8±0.3	0.167[a]
Prothrombin time (%)	89.2±11.8	93.4±15.1	0.209[a]
Platelets ($x10^4/mm^3$)	14.7±8.7	14.1±3.9	0.627[a]
AFP (ng/ml)	15,065±82,251	3,018±18,567	0.267[a]
DCP (mAU/m)[c]	7,011±28,784	7,926±24,508	0.853[a]

Data are expressed as number or mean ± standard deviation. BCAA, branched-chain amino acid; HCC, hepatocellular carcinoma; MTT, molecular targeting therapy; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin. [a]Unpaired t-test; [b]Fisher's exact test; [c]missing data, n=3.

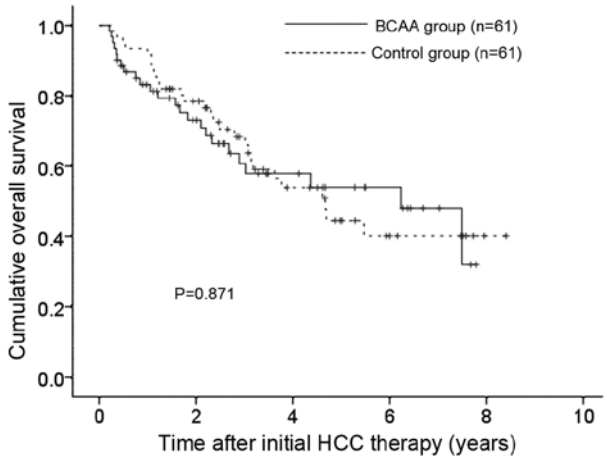

Figure 3. Cumulative overall survival in patients with pretreatment serum albumin level ≥4.0 g/dl (cohort 2). The 1-, 3- and 5-year OS rates after each therapy for HCC were 83.2, 60.7 and 54.0%, respectively, in the BCAA group (n=61) and 91.8, 66.0 and 44.5%, respectively, in the control group (n=61) (P=0.871).

to be significant factors (Table IV). The multivariate analyses involving nine factors with P<0.1 in the univariate analysis showed that HCC stage (P=0.019), ALP ≥300 IU/l (P=0.023) and AFP ≥100 ng/ml (P=0.019) were significant independent predictors linked to OS. The HRs, 95% CI and P-value for nine factors are detailed in Table IV.

Subgroup analysis according to HCC stage. Since HCC stage was an independent predictor associated with OS in both cohort 1 and 2, we further performed subgroup analyses according to HCC stage. In cohort 1 patients, there were 74 patients with HCC stage I or II in the BCAA group and 77 patients with HCC stage I or II in the control group. In terms of OS, no significant difference was observed in the two groups (P=0.353) (Fig. 4A). In cohort 1 patients, there were 37 patients with HCC stage III or IV in the BCAA group and 34 patients with HCC stage III or IV in the control group. In terms of OS, the difference in the two groups reached significance (P=0.017) (Fig. 4B). On the other hand, cohort 2 patients included 32 and 29 patients with HCC stage I or II and III or IV in the BCAA group and 34 and 27 patients with HCC stage I or II and III or IV in the control group. Regardless of HCC stage, no significant difference was found in terms of OS (P=0.785 for HCC stage I or II and P=0.572 for HCC stage III or IV) (Fig. 5).

Discussion

BCAA granules have a variety of pharmacological effects. Kawaguchi *et al* (8,35) and Kawaguchi and Sata (34) showed that BCAA granules can improve albumin synthesis, insulin resistance, immune function and patients' QOL while they can reduce liver related complications and occurrence of HCC. In addition, BCAA supplementation can help in the management of HCC patients since most HCC patients have underlying LC

Table IV. Univariate and multivariate analysis of factors contributing to overall survival in HCC patients with pretreatment serum albumin level ≥4.0 g/dl (cohort 2).

Variables	n	Univariate analysis P-value[a]	Multivariate analysis	
			Hazard ratio (95% CI)	P-value[b]
Gender, male vs. female	85/37	0.422		
Age (years), ≥72 vs. <72	60/62	0.880		
BCAA vs. control	61/61	0.871		
HCC stage I or II vs. III or IV	66/56	<0.001	0.437 (0.219-0.872)	0.019
Maximum tumor size (cm), ≥4.0 vs. <4.0	58/64	<0.001	0.610 (0.307-1.213)	0.159
Cause of liver disease, viral vs. non-viral	80/42	0.517		
AST (IU/l), ≥50 vs. <50	61/61	0.024	0.679 (0.307-1.502)	0.339
ALT (IU/l), ≥40 vs. <40	65/57	0.058	0.486 (0.210-1.127)	0.093
ALP (IU/l), ≥300 vs. <300	62/60	<0.001	0.466 (0.241-0.902)	0.023
GGT (IU/l), ≥80 vs. <80	61/61	0.009	0.579 (0.299-1.122)	0.105
Serum albumin level (g/dl), ≥4.3 vs. <4.3	49/73	0.456		
Total bilirubin (mg/dl), ≥1.0 vs. <1.0	40/82	0.045	0.897 (0.465-1.730)	0.746
Platelet count ($x10^4/mm^3$), ≥13 vs. <13	65/57	0.372		
Prothrombin time (%), ≥80 vs. <80	102/20	0.937		
Serum AFP (ng/ml), ≥100 vs. <100	48/74	0.013	0.481 (0.260-0.889)	0.019
DCP (mAU/ml), ≥100 vs. <100[c]	73/46	<0.001	0.560 (0.250-1.254)	0.158

BCAA, branched-chain amino acid; HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; CI, confidence interval. [a]Log-rank test; [b]Cox proportional hazard model; [c]missing data, n=3.

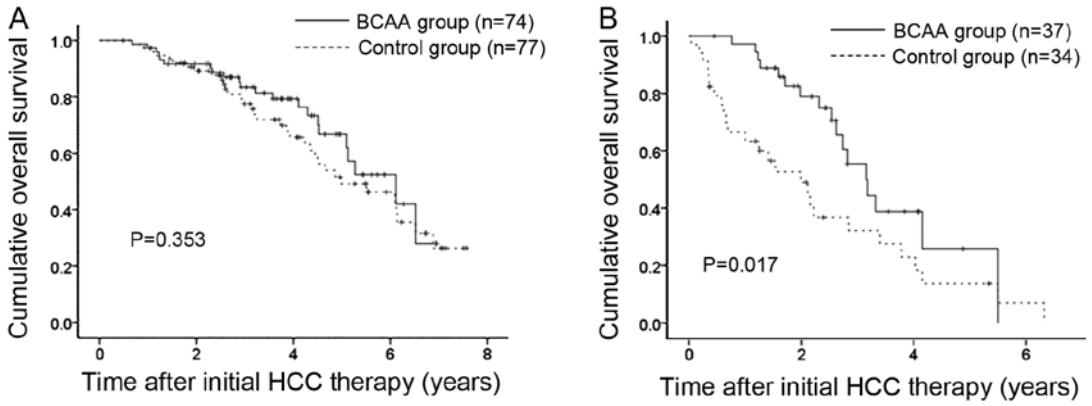

Figure 4. Subgroup analyses in patients with HCC stage I or II (n=74 in the BCAA group and n=77 in the control group) (A) and HCC stage III or IV (n=37 in the BCAA group and n=34 in the control group) (B) in cohort 1 (pretreatment serum albumin level ≥3.6 g/dl and <4.0 g/dl) in terms of OS.

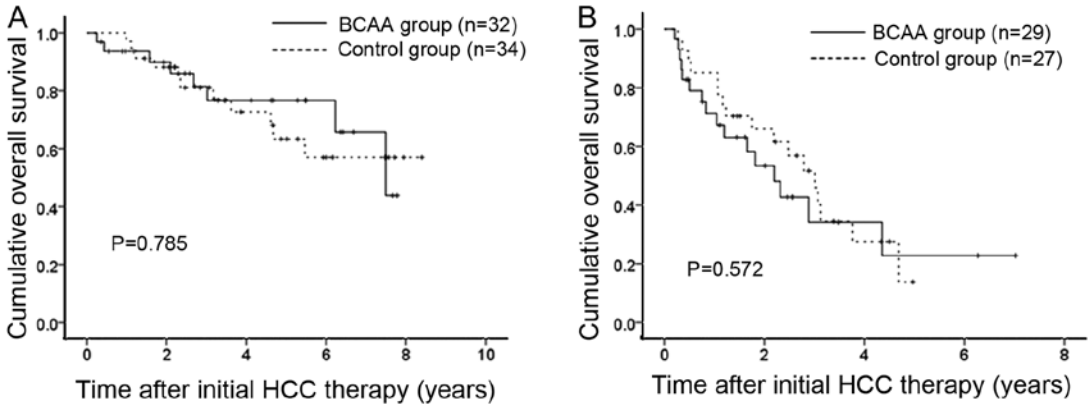

Figure 5. Subgroup analyses in patients with HCC stage I or II (n=32 in the BCAA group and n=34 in the control group) (A) and HCC stage III or IV (n=29 in the BCAA group and n=27 in the control group) (B) in cohort 2 (pretreatment serum albumin level ≥4.0 g/dl) in terms of OS.

(24,27,36,37). However, whether early interventional therapy using BCAA granules in patients with HCC can improve survival remains unclear. An interesting issue is when is the optimal timing of nutritional support such as BCAA granules in patients with HCC. Hence, we conducted these comparative studies.

In our results, in cohort 1 (patients with pretreatment serum albumin level ≥3.6 and <4.0 g/dl), the OS rate in the BCAA group tended to be higher compared to that in the control group and in subgroup analysis in patients with HCC stage III or IV, which means advanced stage of HCC, the OS rate in the BCAA group was significantly higher compared to that in the control group, although in other analyses, no significant difference in the two groups was found. These results suggest that early interventional therapy using BCAA granules can be a treatment option for some selected patients.

In general, in patients with advanced stage HCC, curative therapies are difficult to perform due to tumor characteristics. Hence, repeated therapies for HCC will be needed in these patients. However, these repeated therapies can lead to deterioration of liver functional reserve as reflected by hypoalbuminemia (24,27). BCAA supplementation actually improves hypoalbuminemia. Moreover, Kawaguchi *et al* reported that BCAA granules may suppress hepatic neovascularization and hepatocarcinogenic activity and Yoshiji *et al* demonstrated that BCAA therapy significantly suppressed glucose- and insulin-induced angiogenesis in the presence of vascular endothelial growth factor (VEGF) (8,22). Angiogenesis is a key process in tumor growth and VEGF, which stimulates angiogenesis, appears to be essential for HCC progression (8,22). Our present results may be associated with these observations. On the other hand, advanced malignancy can result in muscle wasting and systemic catabolism, with BCAA treatment having the potential to improve these poor conditions (36).

As described above, in cohort 2 patients (pretreatment serum albumin level ≥4.0 g/d), the difference in the BCAA and control groups did not reach significance in the analyses in terms of OS. Very early intervention using BCAA granules might not be beneficial for improving OS. Curative treatment at the initial therapy for HCC, close surveillance for HCC recurrence and adequate therapy for recurrence will be more important than nutritional therapy in these patients for prolonging OS.

This study included several limitations. First, this is a retrospective observational study although propensity score matching analyses were performed for reducing selection biases in this study. Second, our patient cohorts included heterogeneous patient populations with various stages of HCC

and various causes of underlying liver diseases. Third, BCAA treatment adherence in each individual, antiviral therapies such as interferon therapy for patients with HCV or nucleoside analogue therapy for those with hepatitis B virus during observation period were not included in the present analyses, leading to bias (38-40). Hence, further studies with well selected patient population will be needed. However, our study results demonstrated that early interventional therapy using BCAA granules may be effective in some selected patients.

We concluded that in HCC patients with pretreatment serum albumin level ≥3.6g/dl, early BCAA supplementation can be a treatment option for improving clinical outcome.

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