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Interferon-Induced Prolonged Biochemical Response Reduces Hepatocarcinogenesis in Hepatitis C Virus Infection

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The aim of this study was to elucidate indicator of interferon (IFN) therapy for reducing hepatocellular carcinoma (HCC) in chronic hepatitis C patients without eradicating hepatitis C virus (HCV) RNA during IFN therapy. Inclusion criteria were biopsy-proven chronic hepatitis or liver cirrhosis, IFN period for more than 1.5 years and persistently positive HCV-RNA during IFN therapy. Two hundred thirty-six patients satisfied above criteria were treated with IFN for 1.5–5 years (median 1.8 years, mean 2 years). Mean age was 55.1 years and male was 145(61%). Eighty-one (34%) patients had severe fibrosis of the liver. These patients were prospectively monitored about HCC after the termination of IFN therapy. We regarded biochemical response (BR) as normalization of serum aminotransferase and alpha-fetoprotein for more than 1 year during IFN therapy. Cumulative rate of development of HCC after the termination of IFN therapy was 9.1% at 5th year and 26.5% at 10th year. Cox proportional analysis showed that HCC development after the termination of IFN therapy occurred when histological staging was advanced ($P < 0.0001$) and BR was not achieved ($P = 0.009$), age was >60 years ($P = 0.026$). The relative risk of HCC development in patients with BR was 0.36 compared with patients without BR. The attainment of BR during IFN therapy is effective in reducing hepatocarcinogenesis for patients with chronic HCV infection. ***J. Med. Virol. 79:1485–1490, 2007.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis C; hepatocellular carcinoma; interferon; biochemical response

INTRODUCTION

Current interferon (IFN) therapy for patients with chronic hepatitis C viral infection has been directed at viral clearance. Recent studies reported improvement of therapeutic efficacy when IFN is combined with ribavirin [Schalm et al., 1997; McHutchison et al., 1998, 2000; Poynard et al., 1998; Reichard et al., 1998]. Novel long-acting formulations of IFN known as pegylated IFN induced higher eradication rate of hepatitis C virus (HCV) [Zeuzem et al., 2000; Lindsay et al., 2001; Manns et al., 2001]. However, some patients do not have viral clearance despite these new IFN therapies.

Fortunately, IFN has various actions to protect some malignancies except for action of eradicating HCV [Gutterman, 1994; Fogler et al., 1996; Scarpa et al., 1997; Murphy et al., 2001; Lindner, 2002]. There have been several articles addressing the issue of IFN and its effect on reducing hepatocellular carcinoma (HCC) in hepatitis C even if the patients did not clear HCV RNA [Nishiguchi et al., 1995; Ikeda et al., 2000, 2001]. However, there is also controversy over what patients should

Abbreviations used: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BR, biochemical response; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon.

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be treated or how long the IFN therapy should be continue to protect HCC appearance. For the present, the indicator to decide the period of prolonged IFN therapy for protecting HCC in patients with chronic hepatitis C viral infection is not known.

Thus, the indicator of IFN therapy was evaluated to reduce the development of HCC in patients who were persistently positive for serum HCV-RNA during IFN therapy. This study was a prospective evaluation initiated at the time patients completed IFN treatment.

MATERIALS AND METHODS

Patients

The number of patients who were diagnosed with chronic HCV infection and were subsequently treated a small dose of IFN-monotherapy or intermittent therapy to reduce ALT and hepatocarcinogenesis at the study hospital between April 1991 and March 2003 was 513. Out of 513, 236 patients had the following criteria: (1) laparoscopy and liver biopsy taken within 6 months of initiation of IFN therapy, which showed histopathological features of chronic hepatitis or liver cirrhosis; (2) IFN treatment with 3 or 6 million units (MU) of IFN by injection two or three times a week for more than 1 year; (3) period of IFN therapy was 1.5–5 years; (4) persistently positive for HCV-RNA by amplicor monitor assay [Albadalejo et al., 1998] or reverse transcription nested polymerase chain reaction (RT-nested PCR) [Hagiwara et al., 1992] at 6, 12 months after the initiation of IFN therapy and at the termination of IFN therapy; (5) 45 years and over; (6) average alanine aminotransferase (ALT) elevation greater than 1.5 times the upper normal limits (ALT normal range: 12–50 IU) for more than 6 months before IFN therapy; (7) no treatment with corticosteroid, immunosuppressive agents, ribavirin, or antiviral agents within 6 months of commencement of IFN therapy; (8) negative for hepatitis B surface antigens, antinuclear antibodies, or antimitochondrial antibodies in the serum, as determined by radioimmunoassay and spot hybridization; and (9) Leukocytes $>$ 3,000/mm^3, platelets $>$ 80,000/mm^3, and bilirubin $<$ 2.0 mg/ml before IFN therapy. Patients with either of the following criteria were excluded from the study: (1) alpha-fetoprotein (AFP) of 400 ng/ml or higher, (2) advanced and decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites, (3) a short follow-up period of 6 months or less, (4) IFN was given daily at the first stage of treatment, or (5) combination therapy of IFN and ribavirin.

On the IFN, one group of 155 patients was given natural IFN-alpha (Sumitomo Pharmaceutical Co., Osaka, Japan). Another group of 47 patients received recombinant IFN-alpha-2a (Roferon-A; Hoffmann-La Roche, Tokyo, Japan or Canferon A; Takeda Pharmaceutical Co., Osaka, Japan). A third group of 34 patients received natural IFN-beta (Toray Industries or Daiichi Pharmaceutical Co., Tokyo, Japan). The effects of IFN therapy were divided into four categories as followings: (1) continuous normalization of aminotransferase and AFP for more than 1 year during IFN therapy (normal range, aspartate aminotransferase (AST); 11–38 IU/L, ALT; 6–50 IU/L; and 10 ng/ml, AFP) Biochemical response (BR), (2) continuous normalization of aminotransferase without normalization of AFP, (3) continuous normalization of AFP without normalization of aminotransferase, (4) abnormal aminotransferase and/or AFP excluded the above three categories. The patients who showed normal AFP before and during IFN treatment were categorized as having normalization of AFP. The patients were prospectively monitored the HCC development. The study was approved by the institutional ethics review. Each patient gave informed consent.

Blood Testing

Blood samples were obtained just before IFN therapy and stored at −80°C. Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) [Doglio et al., 1999].

On the other hand, serum HCV-RNA at 6, 12 month after initiation of initial IFN therapy and the termination of IFN therapy were analyzed by the qualitative PCR assay or RT-nested PCR. The lower detection limit of the qualitative assay is 100 copies/ml. HCV genotype was examined by the PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously [Dusheiko et al., 1994]. Serum AST, ALT, and AFP concentrations were measured at least once per month for 6 months prior to the initiation of IFN therapy, once a month during IFN administration and thereafter.

Follow-Up Protocol

The start of this study was defined as the first day of the termination of IFN treatment. Clinical evaluation and biochemical and hematological tests were performed at 1–3 monthly intervals. Fifteen patients were lost to follow-up. Because no HCC appeared in these 15 patients, they were removed as the subject of this study at the time of final consultation in statistical analysis [Harrington and Fleming, 1983]. Deaths unrelated to HCC and patients who started a new treatment in combination with corticosteroids, immunosuppressive agents, or antiviral drugs during the follow-up were also classified as withdrawals and removed the subject of this study at the time of withdrawals. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings on computed tomography and ultrasonography. Microscopic examination of fine-needle biopsy material was carried out in patients whose angiograms did not demonstrate a typical image of HCC.

Liver Histology

Liver biopsy specimens were obtained percutaneously or by peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas. Histopathological interpretation of specimens was made by experienced liver pathologists (YA and HK) who had no clinical information. Baseline liver histology of chronic hepatitis prior to IFN therapy was classified according to the extent of fibrosis, into three stages: mild, periportal expansion; moderate, portoportal septa; and severe, portocentral linkage or bridging fibrosis [Desmet et al., 1994]. Patients with mild or moderate fibrosis were considered as non-severe fibrosis. Patients with severe fibrosis were considered as severe fibrosis (pre-cirrhosis or cirrhosis).

Statistical Analysis

Statistical analysis was the Kaplan–Meier estimate, log rank test, and a cox proportional hazard model where appropriate. A P-value of <0.05 was considered statistically significant. The SPSS software package (SPSS 11.0 for windows; SPSS, Inc., Chicago, IL) was used for analyses. A Cox proportional hazards model was used to analyze the factors contributing to the HCC appearance: factors examined included age, gender, body mass index (BMI), HCV-genotype, HCV RNA level, biochemical factors (AST, ALT, AFP) before IFN therapy and effect after the initiation of IFN therapy, and histological findings.

RESULTS

Patients' Characteristics

Table I shows the characteristics of the 236 patients who had performed IFN therapy. These patients were classified into a severe fibrosis group or a non-severe fibrosis group by histological findings. A total of 81 patients showed a finding of severe fibrosis. Seventy-eight patients showed abnormal serum level of AFP before the initiation of IFN. These 236 patients received IFN for 1.5–5.0 years (median 1.8 years, mean 2 years).

Effect of IFN Therapy for Aminotransferase and AFP

One hundred twenty-one of 236 patients treated with IFN had constant aminotransferase for more than 1 year during IFN therapy. Concerning the serum level of AFP, 28 patients had serum AFP level of >10 ng/ml at the termination of IFN and other patients showed serum AFP level of ≤10 ng/ml. One hundred sixteen patients had BR. Of 120 patients categorized as non-BR, five showed normalization of aminotransferase without normalization of AFP.

Risk Factors for HCC Development After the Termination of IFN Therapy

Cumulative rate of development of HCC after the termination of IFN therapy was 9.1% at 5th year and 26.5% at 10th year. Univariate analysis showed that the following five factors significantly affected the cumulative HCC appearance rate in all the patients: histopathological staging ($P < 0.0001$), BR ($P < 0.0001$), abnormalization of aminotransferase and AFP ($P < 0.0001$), age ($P = 0.001$), AFP before IFN therapy ($P = 0.01$) (Table II). The variables were mutually correlated and multivariate cox regression analysis was performed with the five statistically significant variables in the model. As shown in Table III, three factors (histopathological staging, BR, and age) were associated with rate of HCC development. First, the relative risk for HCC development in patients with severe fibrosis was 5.1 compared with non-severe fibrosis. Second, the relative risk in patients without BR was 2.8 compared with patients with BR. Finally, the relative risk in aged patients with more than 60 years was 2.3 compared with

TABLE I. Clinical Profiles Before the Interferon Treatment

Characteristic	At the initiation of IFN treatment
n[a]	236
Sex (male/female)[a]	145/91
Age (years)[b]	55.1 ± 10.8
Liver fibrosis (non-severe fibrosis/severe fibrosis)	155/81
Genotype (1/2/3)[a]	182/36/3
HCV-RNA (KIU/ml)[c]	980 (76 to >5,000)
AST (IU/L)[b]	80.3 ± 58.3
ALT (IU/L)[b]	123.0 ± 96.4
AFP (ng/ml)[c]	8 (1–190)
WBC ($\times 10^3/mm^3$)[b]	4.7 ± 1.4
Hb (g/dl)[b]	14.4 ± 1.4
Platelet ($\times 104/mm^3$)[b]	14.7 ± 5.5
Administration period of IFN (years)[b]	2.0 ± 2.6

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin; HCV, hepatitis C virus; normal reference ranges 6–50 IU/L for ALT and 11–38 IU/L for AST.
[a]Data are number of patients.
[b]Data expressed as mean ± standard deviation.
[c]Data are median (range).

TABLE II. Factors Associated With Development of HCC After the Termination of IFN Treatment by Univariate Cox Regression Analysis

Factor	Category	Hazards ratio	95% Confidence interval	*P*-value
Liver fibrosis[a]	Non-severe fibrosis	1		<0.0001
	Severe fibrosis	5.43	2.70–10.91	
Normalization of aminotransferase and AFP (BR)[b]	+	1		<0.0001
	–	4.43	2.05–9.59	
Abnormalization of aminotransferase and AFP[b]	+	4.37	1.93–9.92	<0.0001
	–	1		
Age (years)[a]	≤60	1		0.001
	>60	3.31	1.66–6.58	
AFP (ng/ml)[a]	≤10	1		0.010
	>10	2.42	1.23–4.75	
Normalization of AFP without the normalization of aminotransferase[b]	+	1	0.97–4.02	0.061
	–	1.98		
Administration period of IFN (years)	≤2	1	0.19–1.10	0.081
	>2	0.46		
Sex[a]	Male	1	0.35–1.48	0.366
	Female	0.72		
Normalization of aminotransferase without the normalization of AFP[b]	+	1	0.13–2.36	0.557
	–	0.56		
HCV genotype[a]	1	1	0.33–2.22	0.751
	2	0.86		
BMI[a]	≤25	1	0.08–7.29	0.800
	>25	0.74		
ALT (IU/L)[a]	≤100	1	0.53–2.00	0.922
	>100	1.04		
HCV-RNA (KIU/ml)[a]	>100	1	0.42–2.52	0.948
	≤100	1.03		

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BMI, body mass index; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon.
[a]Clinical and laboratory data before IFN therapy.
[b]Laboratory data for more than 1 year during IFN therapy, BR = continuous normalization of aminotransferase and AFP for more than 1 year during IFN therapy, normalization of aminotransferase = continuous normalization of aminotransferase for more than 1 year during IFN therapy, and normalization of AFP = continuous normalization of AFP for more than 1 year during IFN therapy.

patients with 60 and less than 60 years. In Figure 1, the cumulative rates of development of HCC were shown based on the difference of the effect of IFN therapy.

DISCUSSION

The present study was limited by non-randomized controlled trial. Another limitation of the study was that patients were treated with different types of IFN (one of two types of IFN alpha or IFN beta) at different frequencies (two or three times weekly) for different duration (1.5–5 years). This heterogeneity makes it slight difficult to interpret the results of the study.

However, the present study shows several findings to decide the period of prolonged IFN therapy for protecting HCC in patients without eradicating HCVRNA. First, on the cumulative HCC development rate after the termination of IFN, patients with BR had low cumulative HCC rate compared to those without BR. Even if serum HCV RNA is positive during IFN therapy,

TABLE III. Factors Associated With Development of HCC After the Termination of the IFN Therapy by Multivariate Cox Regression Analysis

Factor	Category	Hazards ratio	95% Confidence interval	*P*-value
Liver fibrosis[a]	Non-severe fibrosis	1	2.36–10.96	<0.001
	Severe fibrosis	5.09		
Normalization of aminotransferase and AFP (BR)[b]	+	1	1.30–6.02	0.009
	–	2.80		
Age (years)[a]	≤60	1	1.10–4.61	0.026
	>60	2.25		

[a]Clinical and laboratory data before IFN therapy.
[b]Laboratory data during IFN therapy, BR = continuous normalization of aminotransferase and AFP for more than 1 year during IFN therapy.

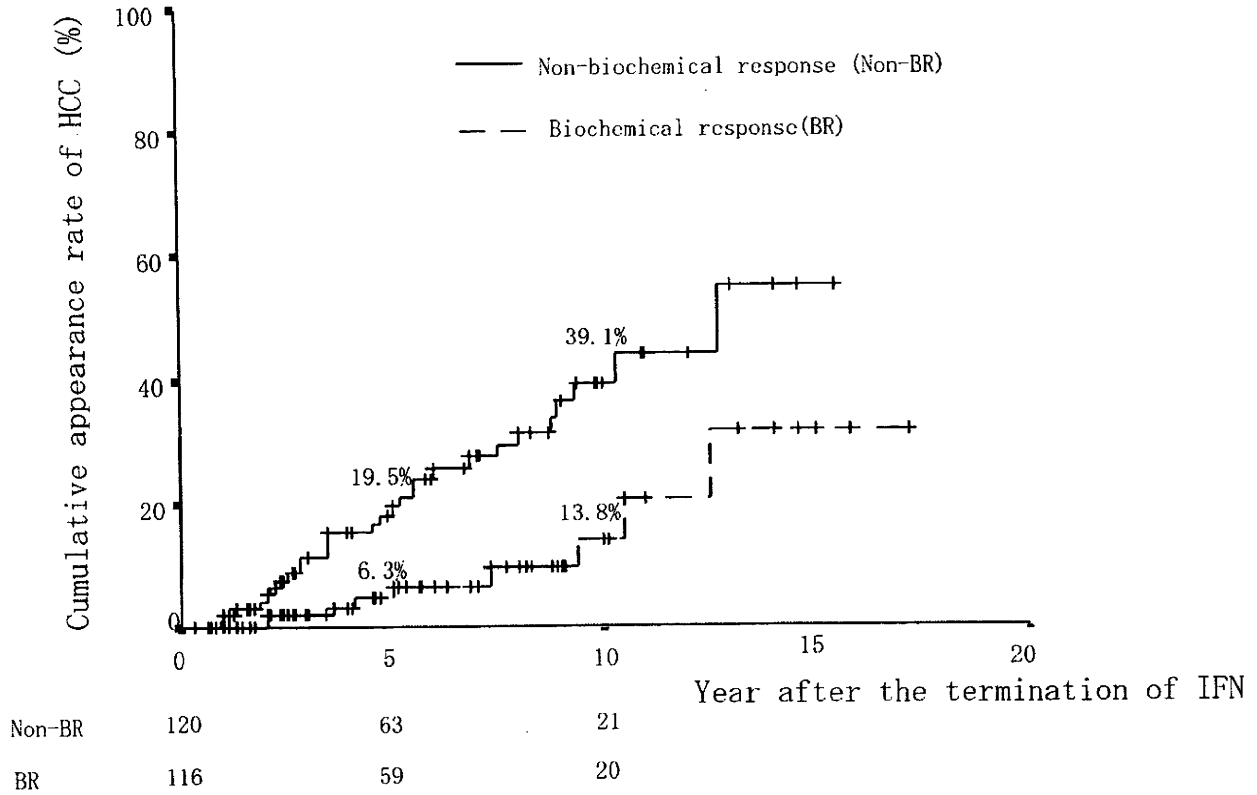

Fig. 1. Cumulative appearance rate of hepatocellular carcinoma (HCC) after the termination of IFN therapy based on the difference of biochemical response.

attainment of BR for more than 1 year during IFN therapy is useful for reducing HCC development. This suggests that both aminotransferase and AFP are suitable indicators in long-term IFN therapy for prohibiting HCC. Second, HCC significantly developed in aged-patients with severe fibrosis. The second finding was almost the same as those of earlier researchers [Imai et al., 1998; Kasahara et al., 1998].

With respect to treatment for chronic hepatitis C, normalization of ALT as well as clearance of HCV-RNA is important. Previous studies have indicated the following: (1) The incidence of HCC was lower in patients with normal ALT levels than in patients with abnormal ALT levels [Ikeda et al., 2001] and (2) prolonged IFN therapy allowed patients to achieve normalization of aminotransferase after IFN therapy most significantly even if serum HCV RNA was positive [Arase et al., 1999]. Thus, apart from eradication of the virus, patients with normalization of ALT levels have a lower possibility of HCC appearance than patients showing elevated ALT after IFN therapy. It is possible that IFN intermittent therapy improves serum ALT levels in patients with chronic HCV infection [Arase et al., 2001]. Improvement of inflammation in liver infected HCV could have the anti-carcinogenesis [Arase et al., 1997].

Another point is that elevated AFP is associated with the hepatocarcinogenesis. AFP has the following functions; First, AFP plays a role in orienting cells toward multiplication or differentiation [Parmelee et al., 1978; Deutsch, 1991]. Second, AFP could control the cell growth. AFP has been found to regulate the proliferation of human mammary tumor cells [Wang and Alpert, 1995]. Third, AFP has been found to have immunosuppressive activity [Lester et al., 1978; Lu et al., 1984]. These results suggest that elevation of AFP enhance carcinogenesis in the patients with chronic HCV infection.

Recent studies reported improvement of therapeutic efficacy when IFN is combined with ribavirin for chronic hepatitis C. However, some patients do not have viral clearance despite these new IFN therapies. Combination therapy with IFN and ribavirin has many side effects. Some patients cannot receive combination therapy of IFN and ribavirin. There is, therefore, a pressing need to develop an effective strategy for the treatment of these patients. The present study suggests that IFN-induced BR reduces hepatocarcinogenesis in HCV infection.

However, long-term IFN treatment can be associated with serious side effects and is costly. Accordingly, careful selection of patients for long-term IFN therapy is extremely important.

In conclusion, the attainment of BR during IFN therapy is effective in reducing hepatocarcinogenesis for patients with chronic HCV infection.

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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.

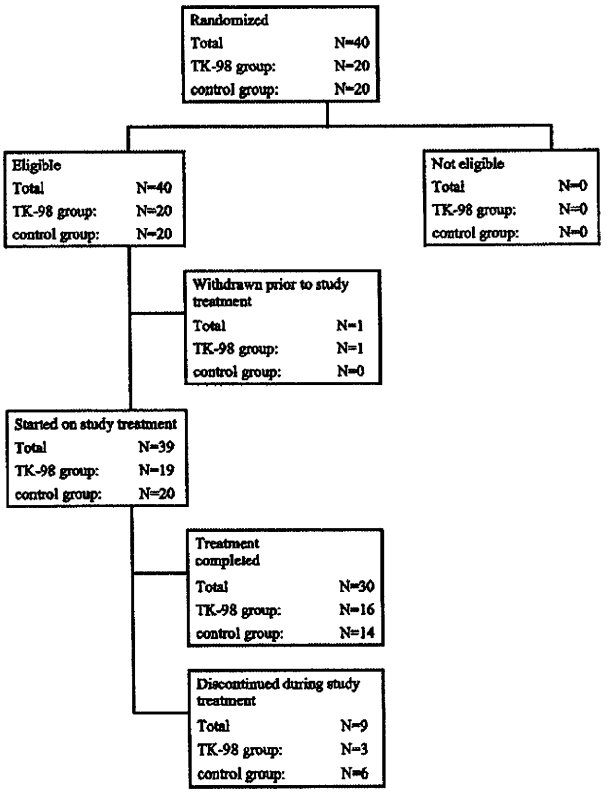

Fig. 1. Flow chart of patients. A total of 39 subjects who initiated study treatment were included in the full analysis set (FAS) and the per protocol set (PPS), as well as a safety analysis

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)

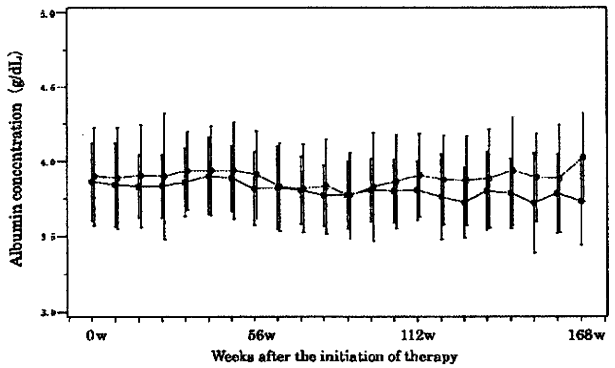

Fig. 2. Serum albumin concentration in TK-98 group (*black dots*) and control group (*white dots*). Data are means; bars show the standard deviation

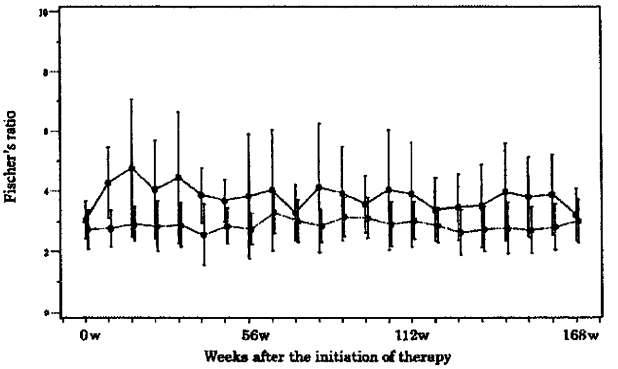

Fig. 3. Fischer's ratio in TK-98 group (*black dots*) and control group (*white dots*). Data are means; bars show the standard deviation

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).

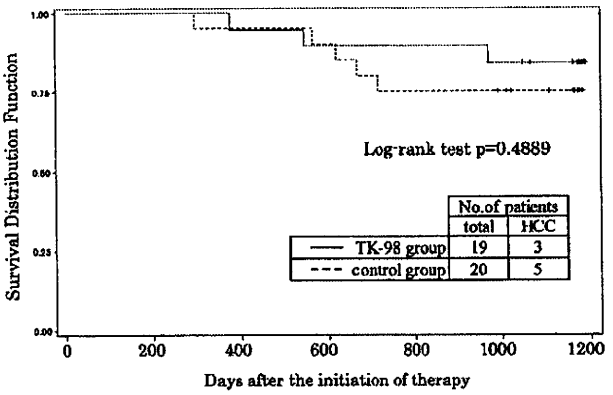

Fig. 4. Kaplan-Meier estimates of event-free survival for hepatocellular carcinoma (*HCC*) in patients with compensated liver cirrhosis caused by hepatitis C virus (HCV) infection

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.

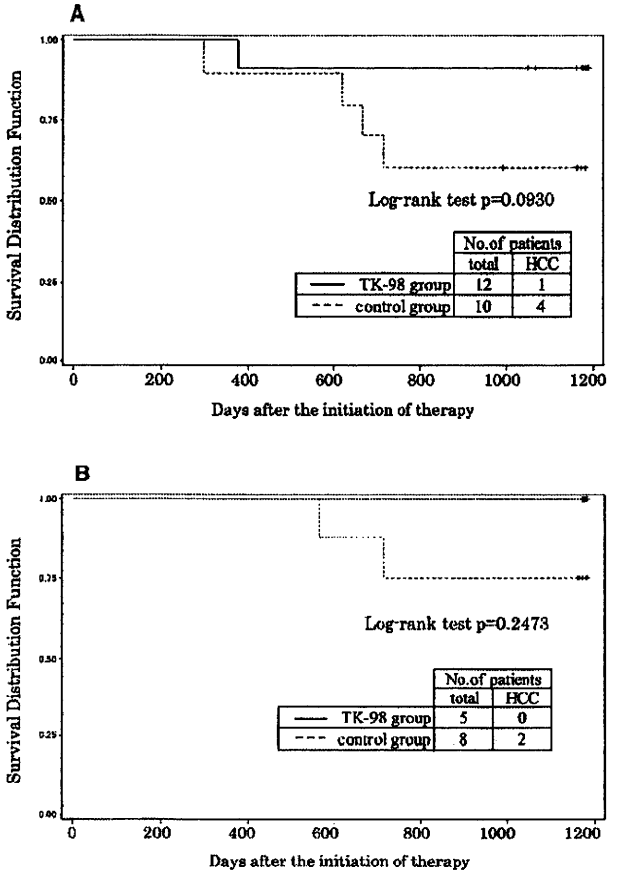

Fig. 5A,B. Kaplan-Meier estimates of event-free survival for HCC in patients with compensated liver cirrhosis caused by HCV infection. **A** Subgroup with a baseline serum albumin level of <4.0 g/dl; **B** subgroup with baseline body mass index (*BMI*) ≥ 25

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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The Efficacy of Short-Term Interferon-Beta Therapy for Type C Cirrhotic Patients with Genotype 2a and Low Virus Load

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The Efficacy of Short-Term Interferon-Beta Therapy for Type C Cirrhotic Patients with Genotype 2a and Low Virus Load

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Abstract

Objective The aim of this study was to elucidate the efficacy of short-term interferon (IFN) therapy for type C cirrhotic patients with genotype 2a and low virus load.

Methods The present study was retrospective cohort study. Inclusion criteria were liver cirrhosis, hepatitis C virus (HCV) genotype 2a, the serum HCV RNA level of less than 100 KIU/mL, and IFN period of 6 or 8 weeks. Twenty-five consecutive patients who satisfied the above criteria were treated with IFN-beta daily at the dosage of 6 MU for 6 or 8 weeks. Independent factors that might have influenced sustained virologic response (SVR) were studied using multiple logistic regression analysis.

Results Background of clinical profiles were as follows: median (range) age=64 (53-76) years, male/female=13/12, and median (range) HCV-RNA=31 (8-90) KIU/mL. Out of 25, 14 patients (56.0%) had SVR by the intention-to-treat analysis. The SVR was significantly associated with serum HCV RNA level. Logistic analysis showed that SVR occurred when HCV RNA level was <50 KIU/mL (p=0.047). Based on the difference of the serum HCV RNA level, the SVR rate was 68.4% (13/19) in patients with a serum HCV RNA level of <50 KIU/mL and 16.7% (1/6) in patients with a serum HCV RNA level of ≥50 KIU/mL.

Conclusions The 6 or 8-week IFN-beta therapy is a possible selection of therapy for cirrhotic patients with HCV genotype 2a and a serum HCV RNA level of <50 KIU/mL.

Key words: liver cirrhosis, hepatitis C virus, genotype 2a, low virus load, interferon, sustained viral response

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Introduction

Current interferon (IFN) therapy for patients with chronic hepatitis C viral infection has been directed at viral clearance. Recent studies have reported improvement of therapeutic efficacy when IFN is combined with ribavirin (1-8). However, IFN is expensive and has a number of serious side effects. Therefore, if the treatment period would become shorter, it could be preferable.

On the other hand, several predictive factors of sustained viral response (SVR) to IFN have been identified, and these include short duration of disease, young age, absence of liver cirrhosis, genotype 2a, low hepatitis C virus (HCV)-RNA levels, HCV and mutant type of nonstructual5A region (9-15). Patients with liver cirrhosis (LC) have a high development of hepatocellular carcinoma (HCC) and progression to decompensated state. Thus, patients with a cirrhotic state should be treated for protection of progression of LC stage. In particular, LC patients with genotype 2a and low HCV-RNA levels might have the possibility of eradication of HCV RNA with a small dose or a short period of interferon (IFN). However, there is also controversy over how long the IFN therapy should be continued to eradicate HCV RNA in

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Table 1. Clinical Characteristics before Short-term Interferon Therapy in Type C Liver Cirrhosis with Genotype 2a and Low Virus Load

Characteristics	(n=25)
Age (years old)*	64 (53-76)
Male/female†	13/12
Period of IFN therapy (6w/8w)†	19/6
Total dose of IFN (MU)*	246 (123-336)
HCV load (KIU/mL)*	31 (8-90)
AST (IU/L)*	83 (39-203)
ALT (IU/L)*	74 (27-412)
Hemoglobin (g/dL)*	12.6 (9.7-16.3)
Platelet(10^4/mm^3)*	11.4 (8.0-17.0)
WBC(10^3/mm^3)*	3.8 (3.0-6.9)

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HCV: hepatitis C virus, IFN: interferon, MU: million unit, WBC: white blood cell count.

*Data are expressed as median (range), †Data are number of patients.

LC patients with genotype 2a and low HCV-RNA.

Thus, in this study, we evaluated the efficacy of short-term interferon (IFN) therapy for type C cirrhotic patients with genotype 2a and a low virus load.

Materials and Methods

Patients

A total of 25 consecutive cirrhotic type C patients treated with IFN-beta for HCV RNA clearance at Toranomon Hospital in Tokyo, Japan between 2002 and 2006 were enrolled in this study. This study was a retrospective cohort study. Enrollment criteria were: repeated alanine aminotransferase (ALT) elevation of greater than the upper normal limits (ALT normal range: 12-50 IU/L) for more than six months; histological evidence of liver cirrhosis at the time of entry into the trial by the use of distinction equation between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection (16); positive serum HCV RNA; serum HCV RNA level of less than 100 KIU/mL; genotype 2a. We excluded from the study all the patients: 1) with concurrent hepatitis B virus (HBV); 2) with a history of IFN therapy; 3) Leukocytes <3,000/mm^3, platelets <80,000/mm^3 and bilirubin >1.5 mg/mL before IFN therapy.

Twenty-five patients received IFN at a dose of 6 million units (MU) of natural IFN-beta (Toray Industries or Daiichi Pharmaceutical Co., Tokyo, Japan) daily for 6 or 8 weeks. In general, patients were treated with IFN for 6 weeks and six patients who were treated for 8 weeks were assigned by randomized controlled trial. We regarded sustained virologic response (SVR) to therapy as clearance of HCV RNA by amplicor method (17) for more than 6 months after cessation of therapy. Our study was approved by the institutional ethics review board of our hospital. The physician in charge explained the purpose and method of the clinical trial as well as the potential adverse reactions to each patient, who later gave his/her informed consent for participation.

Blood testing

Blood samples were obtained just before IFN therapy and stored at -80℃. Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems, USA) (18).

On the other hand, serum HCV-RNA at 6 months after the termination of IFN therapy was analyzed by the qualitative PCR assay. The lower detection limit of the qualitative assay is 100 copies/mL. HCV genotype was examined by the PCR assay, using a mixture of primers for the six sub-

Table 2. Predictive Factors for SVR in Short-term Interferon Therapy in Type C Liver Cirrhosis with Genotype 2a and Low Virus Load

Factor	Category	Odds ratio	95% CI	p value*
HCV RNA (KIU/mL)	<50 /≥50	1/0.09	0.01-0.97	.047
AST (IU/L)	≥76 /<76	1/0.46	0.18-1.17	.102
Age (years)	<60/ ≥60	1/0.22	0.04-1.42	.112
Platelet(10^4/mm^3)	<10/≥10	1/3.00	0.57-15.76	.306
WBC(10^3/mm^3)	<4/≥4	1/2.33	0.46-11.81	.367
Sex	Male /Female	1/0.71	0.14-3.58	.682
ALT (IU/L)	<100/≥100	1/0.75	0.13-4.29	.746
Total dose of IFN (IU/L)	<200/≥200	1/1.29	0.23-7.05	.772
Period of IFN therapy (week)	6/ 8	1/1.19	0.20-6.99	.851

ALT: alanine aminotransferase, AST: aspartate aminotransferase, CI: confidence interval, HCV: hepatitis C virus, IFN: interferon, WBC: white blood cell count.

*p value calculated by logistic regression analysis.

types known to exist in Japan, as reported previously (19).

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test. Independent factors that might have influenced SVR were studied using multiple logistic regression analysis, and the following variables were evaluated as prognostic factors: sex, age, HCV RNA level, liver histology, biochemical factors (AST (aspartate aminotransferase), ALT) before IFN therapy and methods of IFN administration. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Results

Patients' characteristics

Table 1 shows the characteristics of the 25 patients who had performed IFN therapy. Clinical profiles were as follows: median (range) age=64 (53-76) years, male/female= 13/12, and median (range) HCV-RNA=31 (8-90) KIU/mL. All the patients were categorized as Child-Pugh-Turcotte score class A. Of the 25 patients originally included in this study, in five patients the dose of the IFN therapy was reduced from 6 MU to 3 MU because of general fatigue and thrombocytopenia at the time of 1-3 weeks after the initiation of IFN. Thus, the total dose of IFN was 228.0±79.2 million units (MU). The median (range) leukocyte and platelet count in patients with dose reduction were 3.400 (3.100-4.800)/mm^3 and 95.000 (8.8-11.4)/mm^3, respectively, while those in patients without dose reduction were 4.600 (3.000-6.900)/mm^3 and 120.000 (80.000-120.000)/mm^3. Both leukocyte and platelet count in patients without dose reduction were higher than those in patients with dose reduction (leukocyte; p=0.013, platelet; p=0.011).

Efficacy of treatment

Out of twenty-five patients enrolled on present study, 14 patients (56.0%) had SVR by the intention-to-treat analysis.

Table 3. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR

	SVR	Non-SVR	p value
Age (years old) † (<60/≥60)	7/7	2/9	0.183
Sex (male/female) †	8/6	5/6	0.647
Period of IFN therapy† (6 week/8 week)	9/5	8/3	0.986
Total dose of IFN (MU) † (<200/≥200)	5/9	5/6	0.698
HCV-load (KIU/mL) *	16 (8-69)	66 (23-98)	0.021
AST (IU/L) *	63 (39-203)	85 (53-141)	0.730
ALT (IU/L) *	75 (27-434)	88 (34-230)	0.557
Hemoglobin (g/dL) *	13.7 (10.1-16.3)	11.7 (9.7-16.1)	0.139
Platelet(10^4/mm^3) *	12.2 (8.7-17.0)	10.0 (8.0-16.0)	0.096
WBC(10^3/mm^3) *	4.0 (3.1-6.9)	3.8 (3.0-5.3)	0.841

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HCV: hepatitis C virus, IFN: interferon, MU: million unit, SVR: sustained virologic response, WBC: white blood cell count.

*Data are expressed as median (range), †Data are number of patients,

‡p value calculated by the Mann-Whitney U test.

The SVR was significantly associated with serum HCV RNA level. The patients with a HCV RNA level of <50 KIU/mL tend to have high SVR compared to those with higher than that in patients with HCV RNA level of ≥50 KIU/mL (Table 2). Based on the difference of serum HCV RNA level, the SVR rate was 68.4% (13/19) in patients with a serum HCV RNA level of <50 KIU/mL and 16.7% (1/6) in patients with a serum HCV RNA level of ≥50 KIU/mL. Table 3 shows the differences in the clinical background between patients with SVR and those without SVR. The serum level of HCV RNA in patients with SVR was lower than that in patients without SVR.

Adverse events

Within one week after the initiation of treatment, flu-like symptoms appeared in all the patients. The leukocyte count was 4,320±1,370/mm^3 and the platelet count was 119,000±23,000/mm^3 before the initiation of IFN therapy, whereas the values were 2,670±830/mm^3 and 71,000±17,000/mm^3, respectively, two weeks after the initiation of the therapy. None of the patients withdrew from this treatment due to IFN-related side effects.

Discussion

The present study was limited by non-randomized controlled trial. Another limitation of the study was that the number of the patients was small. However, several findings from the present study have direct implications for the short-term IFN treatment of LC patients with genotype 2a and low virus load.

First, more than 50% of patients cleared HCV RNA. This result indicates that the 6- or 8-week regimen of IFN therapy was preferable to eradicate HCV RNA in LC patients with genotype 2a and low virus load. Second, the patients with HCV RNA level of <50 KIU/mL tend to have high SVR compared to those with higher than that in patients with HCV RNA level of ≥50 KIU/mL. On the treatment

period, the efficacy of the 6-week regimen of IFN therapy was almost the same as that of the 8-week regimen. Moreover, the efficacy of the total dose of IFN of <200 MU was not different from that by the total dose of ≥200 MU. These results indicate that in about two-thirds of LC patients with a genotype 2a and serum HCV RNA level of <50 KIU/mL and low virus load, HCV was eradicated by the 6-week regimen or total dose of IFN of <200 MU.

Regarding the side effects of IFN, no patient withdrew the treatment due to IFN-related side effect. Okanoue et al (20) reported that side effects occurred when the daily IFN dose was increased. In the present study, five patients had to reduce the IFN dose due to IFN side effects. On the IFN therapy for LC patients, the physician in charge should check the clinical findings compared to the patients with chronic hepatitis C.

At present, the combined IFN and ribavirin therapy is a standard therapy for chronic hepatitis C patients with genotype 1b and a high load of HCV-RNA. However, prolonged combination therapy of IFN and ribavirin is associated with various side effects. If the total dose of IFN is decreased and the period of IFN therapy is short, it would be desirable from two points of cost and side effect. Fortunately, in patients with low HCV-RNA levels, HCV RNA tends to be eradicated with a small dose of IFN (21-24). The present study indicates that in patients with a low HCV-RNA, HCV RNA can be eradicated with a small dose of IFN.

Conclusion

The present study indicates that the 6 or 8-week of IFN therapy is a possible selection of therapy for liver cirrhotic type C patients with genotype 2a and low virus load.

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