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BASIC STUDIES

New ablation procedure for a radiofrequency liver tissue coagulation system using an expandable needle

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Keywords
expandable needle – liver cancer – radiofrequency ablation (RFA) – stepwise expansion

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Abstract

Objective: The stepwise hook extension technique for an expandable needle, which we reported previously, allowed roll-off in short time with low power. The aim of this study was to investigate experimentally the efficacy of a modified extension procedure. *Methods:* Three pigs underwent 10 radiofrequency ablation (RFA) procedures using the 10-hook electrode of LeVeen needle. The conventional technique was used in five RFA (group 1; the electrode was deployed in four steps to full extension), while the new technique was used in the other five RFA (group 2; the electrode was closed after the same three steps as group 1 and then fully extended). *Results:* The shape of the RFA-induced zone was cone-like or irregular in group 1 and oval-like in group 2. The diameter vertical to the shaft was larger in group 2 (37, range 33–42 mm) than in group 1 (23, range 20–29 mm). The median ablation time was longer in group 2 (10 min 13 s) than in group 1 (3 min 56 s). Although the required energy was higher in group 2 than in group 1, that per volume was comparable between the groups (median 0.9 vs. 1.4 kJ/mm^3). *Conclusions:* Our new procedure requires a longer session but produces larger necrosis of a uniform ellipsoid volume, making it potentially suitable for tumours more than 3 cm in diameter.

Percutaneous treatment including radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI) is often used for small-size hepatocellular carcinoma (HCC) as it is less invasive than surgical therapy. RFA has become the first-choice local treatment because of the excellent outcome; the efficacy of RFA in HCC tumours measuring < 2 cm in diameter is similar to that of PEI but it requires fewer treatment sessions, and the efficacy in HCC tumours > 2 cm in diameter is better than with PEI (1). In addition, RFA is also more cost-effective than surgical resection of small HCC (2). Because the volume ablated during one RFA session is of a diameter < 3.0–4.0 cm in most cases, RFA therapy is now restricted to tumour < 3 cm. In this regard, previous studies reported that the necrotic area could be enlarged by a saline injection before RFA (3, 4), combination of RFA with PEI (5, 6), RFA with an ethanol–lipiodol injection (7), RFA with transcatheter arterial embolization (8) and RFA with transient arterial obliteration (9–11).

Among the three commercially available RFA apparatuses, the radiofrequency tumour coagulation system (RTC system; Boston Scientific, Natick, MA, USA), radiofrequency interstitial tumour ablation system (RITA System, RITA Medical Systems Inc., Mountain View, CA, USA) and cool-tip RF system (Radionics Inc., Burlington, VT, USA), the first two types have adopted the expandable needle. We reported previously the efficacy of the stepwise hook extension technique for RFA therapy of HCC (12). The technique allows rapid roll-off at a lower power and reduces any possible increase in intratissue pressure that may cause scattering of intrahepatic metastasis (13–15). Additionally, we have designed a new technique involving full re-expansion after stepwise extension, that may ensure full expansion of the needle to enlarge the ablated zone. The aim of this study was to investigate experimentally the new expansion technique and to compare it with the conventional stepwise extension technique.

Materials and methods

We used the RTC system comprising the RF3000 generator and a slim expandable needle (30 mm,

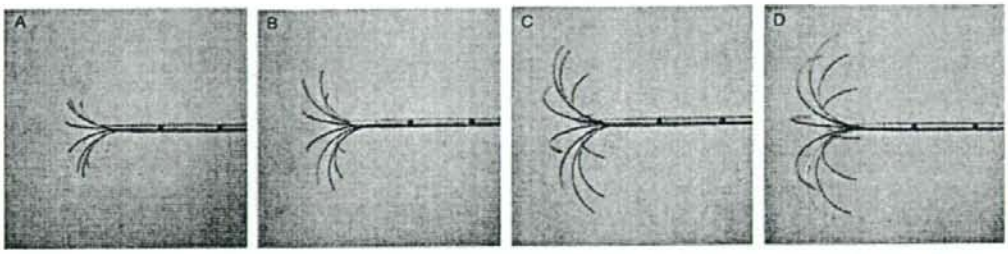

Fig. 1. The conventional four-stepwise procedure. (A) A quarter-length of the electrode tines is expanded in the first step. (B) A half-length is expanded in the second step. (C) A three-quarter length is expanded in the third step. (D) A full-length is expanded in the final step.

17-G LeVeen needle, SuperSlim®), which consists of 10 expandable monopolar array electrodes. RF was applied in the livers of three normal female domestic pigs (weight range: 58–62 kg) under general anesthaesia maintained until killing. The abdomen was opened so that the needle could be inserted directly into the upper region of the liver where the thickness was larger than about 3.5 cm. As a pig liver consists of five thin lobes, RFA sessions were performed four to six times in each liver respectively. Two electrode pads were placed on the skin. Instead of using the standard method recommended by the manufacturer, we adopted two types of stepwise hook extension techniques. In five RFA sessions, the conventional four stepwise expansion was performed (group 1); the electrode tines were expanded to a quarter, a half, three quarters of the length and full length in the first, second, third and final steps: The diameter of the array at each step was 15, 20, 25 and 30 mm, respectively, as shown in Figure 1. In the other five RFA sessions (group 2), the first, second and third steps were similar to those of group 1. After the third step, the tines were again closed within the shaft and then sharply and fully expanded (Fig. 2). The needle extension was observed under an X-ray at each step in the five examples.

Power was first applied at 30 W and then increased at 10 W increments every minute in each step. The necessary electric power and tissue impedance were recorded every 15 s. The procedure was applied continuously until an increase in impedance (caused by coagulation necrosis) with a corresponding decline in delivered power (a phenomenon called 'roll-off').

After completion of the experiments, the animal was killed and the ablated liver lobes were dissected out immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone. We measured the length of the ablated zone along the needle tract and the diameter of the area perpendicular to it. Using these values, we calculated the hypothetical volume of the ablated zone. The energy requirement for ablation was integration of the electric power (W) over the ablation time (s), which could be calculated approximately by summing a product of 15 (s) and the electric power measured every 15 s.

The duration of ablation, required energy and the size of the ablated zone were compared between the two groups using Mann–Whitney's *U*-test. All values are expressed as median. A *P*-value < 0.05 denoted the presence of a statistically significant difference.

The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Toranomon Hospital.

Results

Ablation time and required energy

Roll-off was achieved at each step of ablation in all 10 RFA procedures. The time to reach roll-off at each step and total ablation time are shown in Table 1. In group 1, the median time to reach roll-off at a quarter, a half, three quarters and full expansion was 84 (range 58–180), 49 (27–134), 45 (34–100) and 56 (52–148) seconds respectively. The total ablation time for group 1 was 3 min and 56 s (range 2 min and 56 s to 8 min and 36 s). For group 2, the median time to reach roll-off at each step was 107 (range 70–166), 23 (17–153), 61 (22–108) s, and 6 min and 19 s (4 min and 29 s to 6 min 40 s) respectively. The total ablation time was 10 min and 13 s (range 6 min and 34 s to 12 min and 29 s). These results indicate that the durations of the first step, second step and third step were similar for groups 1 and 2 ($P = 1.000$, 0.421 and 1.000), while that of the fourth step and total session were longer for group 2 than group 1 ($P = 0.008$, 0.032) as shown in Table 2. The energy required for one procedure was

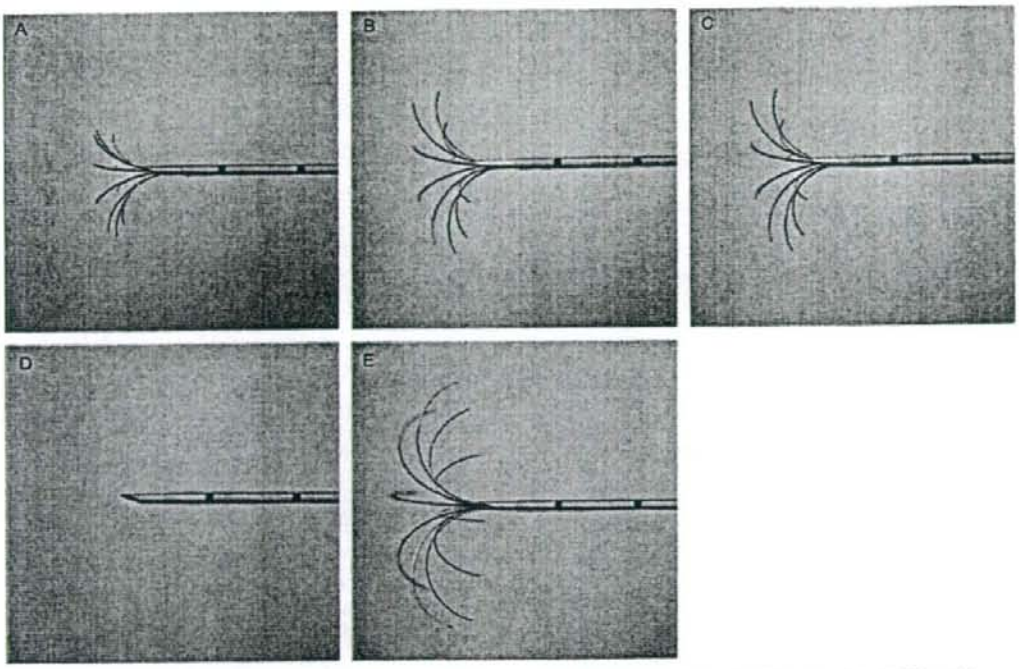

Fig. 2. The new stepwise procedure. (A) A quarter-length of the electrode tines is expanded in the first step. (B) A half-length is expanded in the second step. (C) A three-quarter length is expanded in the third step. (D) The electrode tines are closed in the shaft. (E) Tines are expanded to full length in the final step.

Table 1. Duration of ablation (in minutes seconds) and RF-induced area in groups 1 and 2

	Group 1					Group 2				
	1	2	3	4	5	1	2	3	4	5
Duration										
First step	2′ 55″	3′ 00″	1′ 18″	1′ 24″	0′ 58″	1′ 47″	2′ 15″	2′ 46″	1′ 42″	1′ 10″
Second step	2′ 14″	1′ 28″	0′ 49″	0′ 27″	0′ 32″	1′ 02″	2′ 33″	0′ 18″	0′ 17″	0′ 23″
Third step	0′ 58″	1′ 40″	0′ 35″	0′ 45″	0′ 34″	1′ 48″	1′ 01″	1′ 26″	0′ 22″	0′ 32″
Fourth step	0′ 44″	2′ 28″	1′ 14″	0′ 56″	0′ 52″	5′ 36″	6′ 40″	6′ 19″	6′ 29″	4′ 29″
Total	6′ 51″	8′ 36″	3′ 56″	3′ 32″	2′ 56″	10′ 13″	12′ 29″	10′ 49″	8′ 50″	6′ 34″
RF-induced area										
Transverse diameter, mm	20	28	25	23	22	33	42	38	37	35
Longitudinal length, mm	27	24	30	30	32	20	30	27	27	34
Shape	Irregular	Cone-like	Cone-like	Cone-like	Cone-like	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid

larger in group 2 than that in group 1 (group 1: 5.5 kJ range 4.0–14.8 kJ, group 2: 25.0 kJ range 13.4–30.6 kJ, $P = 0.016$) respectively.

Size and shape of ablated tissue

Table 1 shows the shape and size of the RF-induced areas in groups 1 and 2. In group 1, the shape of the ablated zone was cone-like or was sometimes irregularly shaped. The length along the shaft was longer than the vertical diameter as shown in Figure 3A. In group 2, the ablated zone was near-oval in shape, with the short axis equivalent to the shaft (Fig. 3B). As shown in Table 2, the area perpendicular to the shaft and the ablation volume were larger in group 2 than in group 1: vertical diameter: 23 (range 20–28) mm vs. 37 (range 33–42) mm ($P = 0.008$). This indicates that our technique produced a larger area of necrosis following

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Table 2. Comparison of ablation time (in minutes seconds) and RF-induced areas between groups 1 and 2

	Group 1	Group 2	*P*
Incidence of roll-off	5/5	5/5	1.000
Duration of the first step	1′ 24″ (0′ 58″–3′ 00″)	1′ 47″ (1′ 10″–2′ 46″)	1.000
Second step	49″ (0′ 27″–2′ 14″)	23″ (17″–2′ 33″)	0.421
Third step	45″ (0′ 34″–1′ 40″)	1′ 1″ (22″–1′ 48″)	1.000
Fourth step	56″ (0′ 44″–2′ 28″)	6′ 19″ (4′ 29″–6′ 40″)	0.008
Total ablation time	3′ 56″ (2′ 56″–8′ 36″)	10′ 13″ (6′ 34″–12′ 29″)	0.032
Required energy for ablation, kJ	5.5 (4.0–14.8)	25.0 (13.4–30.6)	0.016
Diameter of the cross-section vertical to the axis, mm	23 (20–28)	37 (33–42)	0.008
Axial length, mm	30 (24–32)	27 (20–34)	0.841
Shape of RF-induced area			
Ellipsoid	0	5	
Cone-like	4	0	
Irregular	1	0	

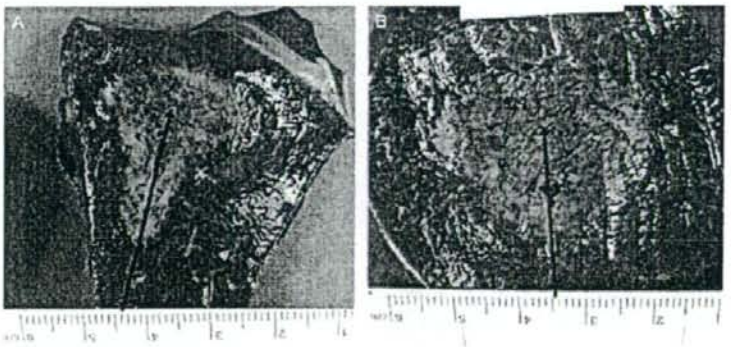

Fig. 3. Photographs of the coagulated area. Arrow shows the direction of the needle shaft. (A) The shape of the area produced by the conventional procedure is cone-like (RFA#3). (B) The shape of the area produced by the new procedure is ellipsoid in shape (RFA#3).

one session of RF. Although the axial length of the ablation zone showed no significant difference between the two groups, that in group 2 seemed a slightly shorter than that in group 1: axial length: 30 (range 24–32) mm vs. 27 (range 20–34) mm ($P = 0.841$). Based on the assumption that the shape of the necrotic area was a combination of a hemisphere and a cone in group 1 and an ellipsoid in group 2, the estimated volume of the ablated liver tissue was 5.7 (range 3.8–7.8) μm^3 for group 1 and 20 (range 11–28) μm^3 for group 2. Using this value and the total required energy for ablation, the calculated energy required for ablation per volume was 0.9 (range 0.7–2.5) J/mm^3 for group 1 and 1.4 (range 0.6–1.8) J/mm^3 for group 2 ($P = 1.000$).

Needle expansion

Figures 4 and 5 show X-ray images of the electrode tines in the pig liver at each step. Both in the second step and the third step, the progress of the tines' spread was smaller than at the first step. The needle expansion at the third step did not reach three quarters length in both groups 1 and 2. The extent of the expansion at the final step was nearly similar to that at the second and third steps in group 1, while it was nearly complete in group 2.

Discussion

Radiofrequency ablation therapy is one of the curative therapies for HCC measuring < 30 mm in diameter, while surgical resection is the only curative treatment for HCC more than 30 mm and < 50 mm in diameter. However, surgical resection cannot be performed in patients with cirrhotic liver and liver dysfunction. Thus, a technique that widens the RF-ablated area can improve, at least theoretically, the survival of cirrhotic patients with HCC over 30 mm in diameter.

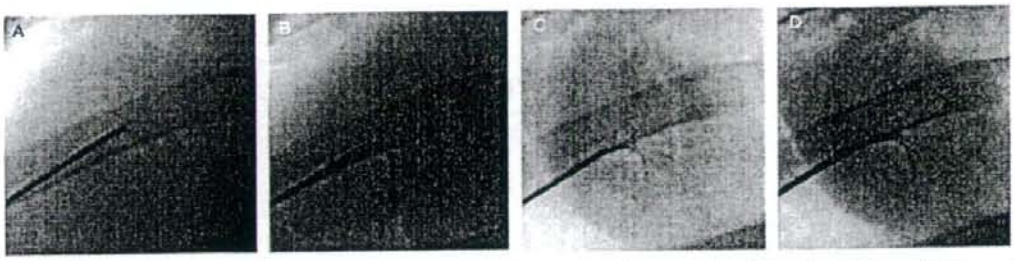

Fig. 4. Electrode tines in a pig liver during the conventional four-stepwise extension procedure. (A) First step. (B) Second step. (C) Third step. (D) Final step. At the second step, the third step and the final step, the progress of the tines' spread is smaller in comparison with that at the first step. The extent of the expansion at the final step was nearly similar to that at the second and third steps.

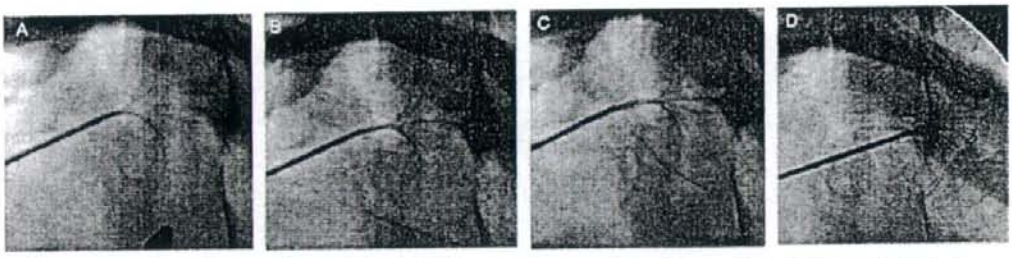

Fig. 5. Electrode tines in a pig liver during the new stepwise extension procedure. (A) First step. (B) Second step. (C) Third step. (D) Final step. At the second step, the third step and the final step, the progress of the tines' spread is smaller in comparison with that at the first step. The extent of the expansion at the final step was nearly complete.

The shape of the ablated zone depends on the needle type (6). For example, the path along the shaft is longer than the transverse diameter when using the cool-tip electrode (Radionics System; Radionics, Burlington, VT, USA), shorter when using the expandable needle of RTC system and compatible with each other when using the LeVeen needle (RTC system). The shorter path is less disadvantageous than the shorter perpendicular diameter, because the ablated zone along with the needle trace can be enlarged by repeating the procedure as the needle is extracted while that perpendicular to the tract cannot be enlarged during one insertion. Although it is often difficult to achieve roll-off during a single-step full expansion procedure using the LeVeen needle, our stepwise procedure has overcome this difficulty and produced an oval ablation zone similar to the single-step procedure.

The LeVeen needle, which had a diameter of 14 G in the first stage, has been made slender for the ease and safety of insertion into the liver. The needle now available in the market has a diameter of 17 G. The slim needle may be easier to deform during insertion and difficult to fully extend within the liver by the conventional stepwise method. The liver tissue resistance consists of resistance acting on the needle tip and that on the side. The strength of the former is proportional to the cross-section and that of the latter is to the surface area. Based on this, the slender shaft is subjected to a large stress and strain resulting in larger deformation, although its resistance is smaller. Thus, the hooks of the slim needle hardly extend as expected; it cannot be fully extended when expanded slowly as shown in Figure 4. This is because the shaft is pushed back as the electrode is inserted towards the liver. To overcome this inconvenience, we investigated a new technique: full re-expansion after stepwise extension, which allows a sharper and definite expansion of the slim needle to full length. Thus, this technique is suggested to be more advantageous in a slimmer needle; this procedure has not been examined in needles 14 or 15 G in diameter.

The additional reason for the larger ablation zone made by the new method is that the tanned tumour or parenchymal tissue would be removed from the surface of the multiple tines when they are once closed in the shaft. The tan was observed on the tip of the shaft

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when the needle was extracted from the liver. The tan adhering on the tine may prevent the uniform electric current, which results in a decrease in the electric efficiency. Thus, the removal of tan can result in an increase in the effectiveness of RF ablation procedure and that in the ablation zone.

A larger ablation zone at the final step of the new technique required a longer coagulation time and a higher input energy during the final step and during the total session; the ablation zone, ablation time and the required energy by our method were larger than those by the conventional stepwise method. The required energy per volume, on the other hand, was almost identical.

In conclusion, the new extension procedure for the expandable needle allows coagulation of larger and more oval area even when using the slim needle. This method may be useful to expand the application of RFA for hepatic tumours.

References

1. Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208–36.
2. Ikeda K, Kobayashi M, Saitoh S, *et al.* Cost-effectiveness of radiofrequency ablation and surgical therapy for small hepatocellular carcinoma of 3 cm or less in diameter. *Hepatol Res* 2005; **33**: 241–9.
3. Livraghi T, Goldberg SN, Monti F, *et al.* Saline-enhanced radio-frequency tissue ablation in the treatment of liver metastasis. *Radiology* 1997; **202**: 205–10.
4. Burdio F, Guemes A, Burdio JM, *et al.* Large hepatic ablation with bipolar saline-enhanced radiofrequency: an experimental study *in vivo* porcine liver with a novel approach. *J Surg Res* 2003; **110**: 193–201.
5. Kurokohchi K, Watanabe S, Masaki T, *et al.* Combined use of percutaneous ethanol injection and radiofrequency ablation for the effective treatment of hepatocellular carcinoma. *Intern J Oncol* 2002; **21**: 841–6.
6. Watanabe S, Kurokohchi K, Masaki T, *et al.* Enlargement of thermal ablation zone by the combination of ethanol injection and radiofrequency ablation in excised bovine liver. *Intern J Oncol* 2004; **24**: 279–84.
7. Kurokohchi K, Masaki T, Miyauchi Y, *et al.* Efficacy of combination therapies of percutaneous on laparoscopic ethanol–lipiodol injection and radiofrequency ablation. *Intern J Oncol* 2004; **25**: 1737–43.
8. Sugimori K, Morimoto M, Shirato K, *et al.* Radiofrequency ablation in a pig liver model: effect of transcatheter arterial embolization on coagulation diameter and histological characteristics. *Hepatol Res* 2002; **24**: 164–73.
9. Yamasaki T, Kurosawa F, Shirahashi H, Kusano N, Hironaka K, Okita K. Percutaneous radiofrequency ablation therapy with combined angiography and computed tomography assistance for patients with hepatocellular carcinoma. *Cancer* 2001; **91**: 1342–8.
10. Kobayashi M, Ikeda K, Kawamura Y, *et al.* Randomized controlled trial for the efficacy of hepatic arterial occlusion during radiofrequency ablation for small hepatocellular carcinoma-direct ablative effects and a long-term outcome. *Liver Int* 2007; **27**: 353–9.
11. Chinn SB, Lee FT Jr., Kennedy GD, *et al.* Effect of vascular occlusion on radiofrequency ablation of the liver: results in a porcine model. *Am J Roentgenol* 2001; **176**: 789–95.
12. Kobayashi M, Ikeda K, Someya T, *et al.* Stepwise hook extension technique for radiofrequency ablation therapy of hepatocellular carcinoma. *Oncology* 2002; **63**: 139–44.
13. Nakamuta M, Kohjima M, Morizono S, *et al.* Comparison of tissue pressure and ablation time between LeVeen and cool-tip needle methods. *Comp Hepatol* 2006; **5**: 10.
14. Kotoh K, Nakamuta M, Morizono M, *et al.* A multi-step, incremental expansion method for radio frequency ablation: optimization of the procedure to prevent increases in intratumor pressure and to reduce the ablation time. *Liver Int* 2005; **25**: 542–7.
15. Kotoh K, Enjoji M, Arimura E, *et al.* Scattered and rapid intrahepatic recurrences after radio frequency ablation for hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 6828–32.

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

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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.

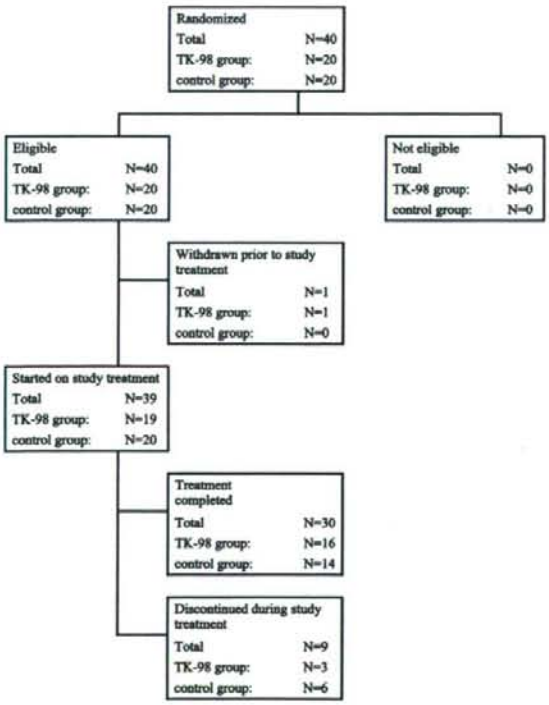

Fig. 1. Flow chart of patients. A total of 39 subjects who initiated study treatment were included in the full analysis set (FAS) and the per protocol set (PPS), as well as a safety analysis

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)*	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
*S/CO score ≥ 10.00 (CLIA method)

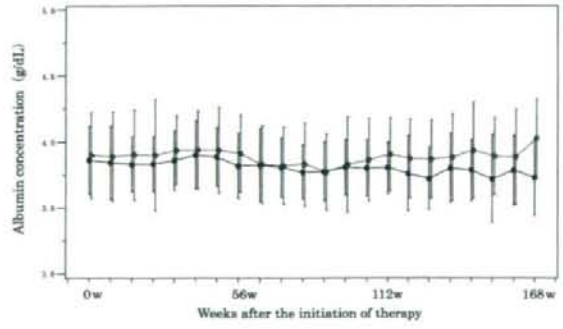

Fig. 2. Serum albumin concentration in TK-98 group (*black dots*) and control group (*white dots*). Data are means; bars show the standard deviation

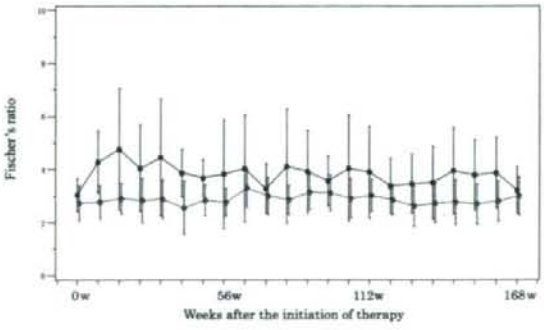

Fig. 3. Fischer's ratio in TK-98 group (*black dots*) and control group (*white dots*). Data are means; bars show the standard deviation

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).

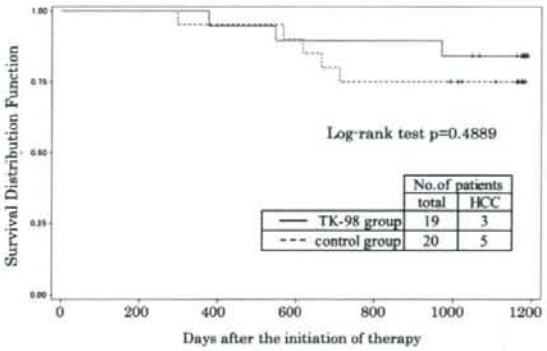

Fig. 4. Kaplan-Meier estimates of event-free survival for hepatocellular carcinoma (*HCC*) in patients with compensated liver cirrhosis caused by hepatitis C virus (HCV) infection

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.

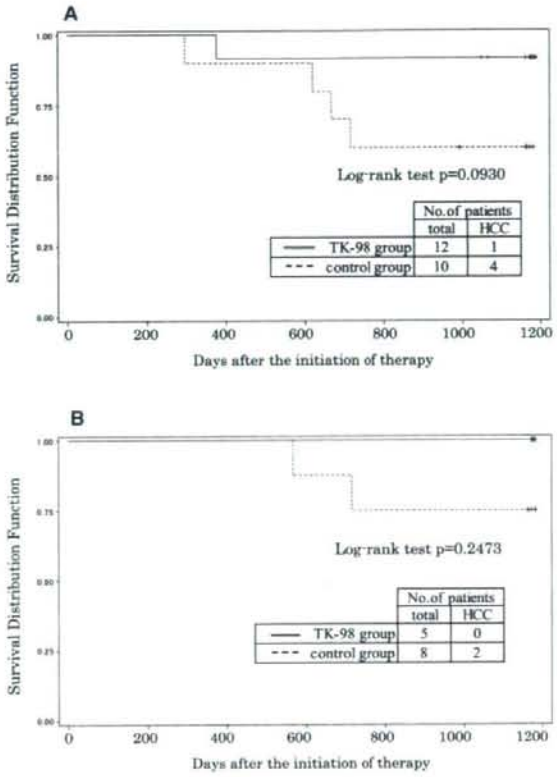

Fig. 5A,B. Kaplan-Meier estimates of event-free survival for HCC in patients with compensated liver cirrhosis caused by HCV infection. **A** Subgroup with a baseline serum albumin level of <4.0 g/dl; **B** subgroup with baseline body mass index (*BMI*) ≥ 25

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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References

1. Yoshida T, Muto Y, Moriwaki H, Yamato M. Effect of long-term oral supplementation with branched-chain amino acid granules on the prognosis of liver cirrhosis. Gastroenterology 1989;24: 692–8.
2. Muto Y, Yoshida T, Sato S, Suzuki K, Watanabe A. Effect of oral supplementation with BCAA-G on the prognosis of liver cirrhosis (in Japanese). Jpn J Parenter Enteral Nutr 1992;14:765–75.
3. Muto Y, Yoshida T, Kato M, Sato S, Suzuki K, Kato A, et al. Serum albumin level and prognosis in patients with liver cirrhosis—result of branched-chain amino acid granules (BCAA-G) supplement trial (in Japanese). Jpn J Parenter Enteral Nutr 1995;17:1135–43.
4. Marchesini G, Bianchi G, Merli M, Amodio P, Panella C, Loguercio C, et al. Italian BCAA Study Group. Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. Gastroenterology 2003;124:1792–801.
5. Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, et al. Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. Clin Gastroenterol Hepatol 2005;3:705–13.
6. Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Koida I, Tubota A, et al. Influence of interferon treatment on carcinogenesis from HCV-related cirrhosis (in Japanese). Igaku to Yakugaku 1998;39: 297–303.
7. Mitamura K, Tamakuma M, Yoshii H. Liver cirrhosis (in Japanese). Igaku-no-Ayumi (J Clin Exp Med) 1999;189:421–5.
8. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology 1998;27:1394–402.
9. Yamada N, Shibata H. A study of the effects of branched chain amino acids in orally administered jelly on severe chronic hepatic

diseases (in Japanese with English abstract). Kitasato Med 1986; 16:268–77.
10. Yoshida T, Katoh M, Moriwaki H. Nutritional assessment and therapy in patients with hepatitis (in Japanese). Kan-Tan-Sui 1989;19:33–40.
11. Habu D, Nishiguchi S, Nakatani S, Kawamura E, Lee C, Enomoto M, et al. Effect of oral supplementation with branched-chain amino acid granules on serum albumin level in the early stage of cirrhosis: a randomized pilot trial. Hepatol Res 2003;25:312–8.
12. Nishiguchi S, Habu D. Effect of oral supplementation with branched-chain amino acid granules in the early stage of cirrhosis. Hepatol Res 2004;30:36–41.
13. Hara K, Yonezawa K, Weng QP, Kozlowski MT, Belham C, Avruch J. Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. J Biol Chem 1998;273:14484–94.
14. Ijichi C, Matsumura T, Tsuji T, Eto Y. Branched-chain amino acids promote albumin synthesis in rat primary hepatocytes through the mTOR signal transduction system. Biochem Biophys Res Commun 2003;303:59–64.
15. Nakamura T, Mori M, Yoshida T, Murakami N, Kato T, Sugihara J, et al. Enzymatic determination of a molar ratio of free branched-chain amino acids to tyrosine (BTR) and its clinical significance in plasma of patients with various liver diseases (in Japanese with English abstract). Rinsho Byori 1989;37:911–7.
16. Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, et al. Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. Hepatol Res 2006;35:204–14.
17. Arao M, Murase K, Kusakabe A, Yoshioka K, Fukuzawa Y, Ishikawa T, et al. Prevalence of diabetes mellitus in Japanese patients infected chronically with hepatitis C virus. Gastroenterology 2003;38:355–60.
18. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625–38.
19. Caldwell SH, Crespo DM, Kang HS, Al-Osaimi AM. Obesity and hepatocellular carcinoma. Gastroenterology 2004;127(5 Suppl 1): S97–103.
20. Oh SW, Yoon YS, Shin SA. Effects of excess weight on cancer incidences depending on cancer sites and histologic findings among men: Korea National Health Insurance Corporation Study. J Clin Oncol 2005;23:4742–54.
21. Moore MA, Park CB, Tsuda H. Implications of the hyperinsulinaemia–diabetes–cancer link for preventive efforts. Eur J Cancer Prev 1998;7:89–107.
22. Balkau B, Kahn HS, Courbon D, Eschwege E, Ducimetiere P. Hyperinsulinemia predicts fatal liver cancer but is inversely associated with fatal cancer at some other sites: the Paris Prospective Study. Diabetes Care 2001;24:843–9.
23. Saito K, Inoue S, Saito T, Kiso S, Ito N, Tamura S, et al. Augmentation effect of postprandial hyperinsulinaemia on growth of human hepatocellular carcinoma. Gut 2002;51:100–4.
24. Nishitani S, Takehana K. Pharmacological activities of branched-chain amino acids: augmentation of albumin synthesis in liver and improvement of glucose metabolism in skeletal muscle. Hepatol Res 2004;30S:19–24.
25. Nishitani S, Takehana K, Fujitani S, Sonaka I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. Am J Physiol Gastrointest Liver Physiol 2005;288: G1292–300.
26. Nishitani S, Matsumura T, Fujitani S, Sonaka I, Miura Y, Yagasaki K. Leucine promotes glucose uptake in skeletal muscles of rats. Biochem Biophys Res Commun 2002;299:693–6.

The Efficacy of Short-term Interferon-beta Therapy for Chronic Hepatitis C Patients with Low Virus Load

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The Efficacy of Short-term Interferon-beta Therapy for Chronic Hepatitis C Patients with 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 chronic hepatitis C patients with low virus load.

Methods The present study was a retrospective cohort study. Inclusion criteria were biopsy-proven chronic hepatitis, the serum hepatitis C virus (HCV) RNA level of less than 100 KIU/ml, IFN period of 8weeks or less. One hundred and eleven consecutive patients satisfied above criteria were treated with IFN-beta (dose: 6 MU, daily for 4, 6, or 8 weeks).

Results Background of clinical profiles were as follows: median (range) age=56 (20-73) years, male/female=64/47, genotype 1b/2a/2b=40/68/3, and median (range) HCV-RNA= 34 (4.5-81) KIU/ml. Out of 111, 64 patients (57.7%) had sustained viral response (SVR). Based on the difference of HCV genotype, the SVR rate was 47.5% (19/40) in genotype 1 and 63.3% (45/71) in genotype 2. In genotype 1, the SVR rate in patients treated with the 8-week-regimen was significantly higher than that in patients treated with the 4- or 6-week regimen. In contrast, in genotype 2, the SVR in patients treated with the 8-week regimen was not significantly different from that in patients treated with the 6-week regimen. None of the patients had severe IFN-related side effects.

Conclusions The 6 or 8-week regiment of IFN-beta therapy is one selection of therapy for chronic hepatitis C patients who have tended to have a SVR and who show IFN-related adverse events.

Key words: chronic hepatitis C, 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 reported improvement of therapeutic efficacy when IFN is combined with ribavirin (1-5). Moreover, novel long-acting formulations of IFN known as pegylated IFN induced higher eradication rate of hepatitis C virus (HCV) (6-8). However, IFN is expensive and has a number of serious side effects. Therefore, if the treatment period becomes shorter, it could be preferable.

Several predictive factors of sustained viral response (SVR) to IFN have been identified, and these include a short duration of disease, young age, absence of liver cirrhosis, low HCV-RNA levels, HCV genotype 2a and mutant type of nonstructural 5A region (9-15). Low dose IFN tends to eradicate HCV RNA in patients who had a low serum level of HCV-RNA. However, there is also controversy over how long the IFN therapy should be continued to eradicate HCV RNA in patients. Thus, in this study we evaluated the duration of IFN therapy in order to eradicate HCV RNA in patients who had low serum levels of HCV-RNA.

Abbreviations: HCV: hepatitis C virus, IFN: interferon, SVR: sustained viral response

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Table 1. Clinical Characteristics before Interferon Therapy in Chronic Hepatitis C Patients*

Characteristics	(n=111)
Age (years old) †	56(20-73)
Male/female ‡	64/47
Liver histology (fibrosis, 1/2/3) ‡	60/25/26
HCV genotype(1b/2a/2b) ‡	40/68/3
HCV load (KIU/ml) †	34 (4.5-81)
AST (IU/L) †	56 (14-226)
ALT (IU/L) †	76 (15-434)

***ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; and HCV, hepatitis C virus.**

† Data are expressed as median(range).

‡ Data are number of patients.

Materials and Methods

Patients

A total of 111 consecutive chronic hepatitis C patients treated with IFN-beta for HCV RNA clearance at Toranomon Hospital in Tokyo, Japan between 1997 and 2006 were enrolled in this study. This study was a retrospective cohort study. Enrollment criteria were: repeated alanine aminotransferase (ALT) elevation greater than the upper normal limits (ALT normal range: 12-50 IU/L) for more than six months; histological evidence of chronic hepatitis within one year of entry into the trial; positive serum HCVRNA; serum HCV RNA level of less than 100 KIU/ml or 1 Meq/ml; genotype 1b, 2a and 2b. We excluded from the study all of 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.

One hundred eleven 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 4, 6 or 8 weeks. In general, patients were treated with IFN for 8 weeks. Eleven patients treated for 4 weeks and thirty patients treated for 6 weeks were assigned by randomized controlled trial. We regarded sustained viral response (SVR) to therapy as clearance of HCV RNA by RT-nested PCR (16) or 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 this 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) (18).

On the other hand, serum HCV-RNA at 6 months after the termination of IFN therapy was 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 (19).

Liver histology

Liver biopsy specimens were obtained percutaneously or by peritoneoscopy using a modified Vim Silverman needle

Table 2. Predictive Factors for SVR in Patients with HCV Genotype 1*

Factor	Category	Odds ratio	95% CI†	p value
Period of IFN therapy (week)	4 or 6/ 8	1/8.93	2.14-37.03	0.003
AST (IU/L)	<76/≥76	1/2.17	0.85-5.55	0.102
Sex	Man / Woman	1/0.56	0.16-2.00	0.367
ALT (IU/L)	<100/≥100	1/1.67	0.47-5.93	0.430
Liver histology (fibrosis)	1 /2,3,4	1/0.79	0.39-1.60	0.507
Age (years)	<50/ ≥50	1/0.80	0.23-2.79	0.726

***ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IFN, interferon and CI; confidence interval.**

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 included more than six portal areas. Histopathological interpretations of these 3-to 4-μm thick sections were made independently by experienced liver pathologists (YA and HK) who had no clinical information or knowledge of chronological order of the biopsies in each pair. The biopsy specimens were scored according to the system of Desmet et al (20).

Statistical analysis

Independent factors that might have influenced SVR were studied using the logistic regression analysis, and the following variables were evaluated as prognostic factors: sex, age, liver histology, biochemical factors (aspartate aminotransferase (AST), ALT before IFN therapy, and period of IFN therapy. Significance of trends in SVR based on periods of IFN therapy was determined Cochran-Armitage trend test. The SPSS software package (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Abbreviations: ALT: alanine aminotransferase, AST: aspartate aminotransferase

Results

Patients' characteristics

Table 1 shows the characteristics of the 111 patients who received IFN therapy. A total of 40 patients showed HCV genotype 1 and the remaining 71 patients showed HCV genotype 2.

Efficacy of treatment

Out of 111, 64 patients (57.7%) had SVR. Based on the difference of HCV genotype, the SVR rate was 47.5% (19/40) in genotype 1 and 63.3% (45/71) in genotype 2. We then investigated the factors associated with SVR after termination of IFN. Univariate analysis in patients with genotype 1 identified the following one factor that influenced SVR when the period of IFN treatment was 8weeks (Table 2). As one factor was associated with SVR, we did not evaluate the multivariate analysis.

On the other hand, univariate analysis in patients with genotype 2 did not identify the factor that influenced SVR (Table 3). In genotype 2, the SVR in patients treated with

Table 3. Predictive Factors for SVR in Patients with HCV Genotype 2 *

Factor	Category	Odds ratio	95% CI†	p value
AST (IU/L)	<76 /≥76	1/2.21	0.80-6.14	0.126
Sex	Man / Woman	1/0.61	0.22-1.64	0.324
Period of IFN therapy (week)	4 or 6/ 8	1/1.63	0.57-4.69	0.361
ALT (IU/L)	<100/≥100	1/1.22	0.41-3.57	0.721
Age (years)	<50/ ≥50	1/0.80	0.23-2.79	0.726
Liver histology (fibrosis)	1 /2,3	1/0.88	0.54-1.70	0.876

***ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; and IFN, interferon and CI; confidence interval.**

Table 4. SVR Based on HCV Genotype and Administration Period of Interferon

HCV genotype	Administration period (week)		
	4W	6W	8W
Genotype 1†	0% (0/6)	33.3% (5/15)	73.7% (14/19)
Genotype 2†‡	40% (2/5)	60% (9/15)	66.7% (34/51)

***HCV indicates hepatitis C virus; and SVR, sustained virological response.**
† p <0.001 in genotype 1, p =0.32 in genotype 2 by Cochran-Armitage method
‡ Three patients had HCV genotype 2b. These three patients were treated for 8 weeks and all the patients showed SVR. Remaining patients had genotype 2a.

the 8-week regimen was similar statistically to that in patients treated with the 4- or 6-week regimen.

Table 4 shows the SVR based on the HCV genotype and period of IFN therapy. According to Cochran-Armitage method, the 8-week IFN therapy regimen was the best in order to eradicate HCV RNA in genotype 1. On the other hand, in genotype 2, the 6-week regimen was almost the same as the 8-week regimen.

Adverse events

Within one week after the initiation of treatment, flu-like symptoms appeared in all the patients. Pain in the joints or muscle occurred in 50 cases. However, none of the patients withdrew from this treatment due to IFN-related side effects.