Table 1 Clinical characteristics at laparoscopy and liver biopsy†

	HBV group	HCV group	P-value
n	1320	2804	
Age	36.6 ± 10.5	51.1 ± 11.6	<0.001
Sex (male percentage)	76.6% (1011)	57% (1597)	<0.001
AST (IU/L)	113.5 ± 154.9	89.5 ± 102.2	<0.001
ALT (IU/L)	194.7 ± 255.7	130.1 ± 145.7	<0.001
Total bilirubin (mg/dL)	0.82 ± 0.45	0.84 ± 0.43	0.553
γ-GTP (IU/L)	64.8 ± 82.9	98.2 ± 83.1	<0.001
Platelet count ($\times 10^4/mm^3$)	15.6 ± 12.6	20.3 ± 17.4	<0.001

†Data are number of patients or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidases; HBV, hepatitis B virus; HCV, hepatitis C virus.

Cumulative appearance rates of HCC

The cumulative development rates of HCC based on the histological findings in the HCV group are shown in Figure 2. In the patients with histological findings of stage 1 or 2, the HCC development rates differed due to differences in the laparoscopic findings. The cumulative development rates of HCC based on the laparoscopic findings in the HCV group are shown in Figure 3. In the patients with laparoscopic findings of an irregular liver surface, the HCC development rates differed due to differences in the histological findings. However, in the patients with laparoscopic findings of a smooth or nodular liver surface, the HCC development rates were not significantly different due to difference in the histological findings.

The cumulative development rates of HCC based on the histological findings in HBV group are shown in Figure 4. In the patients with histological findings of stage 1 or 2, the HCC development rates differed due to difference in laparoscopic findings. However, in patients with stage 3 or 4, HCC development rates were not statistically different in spite of the differences in the laparoscopic findings. The cumulative development rates of HCC based on the laparoscopic findings in the HBV patients are shown in Figure 5. In the patients with the same laparoscopic findings, the HCC development rates did not differ due to differences in the histological findings.

HCC appearance rates based on laparoscopic and histological findings were evaluated by the Cox propor-

Table 2 Relationship between laparoscopic findings and histological stage in patients with chronic type C hepatitis

Laparoscopic finding	Histological stage				
	Stage 1	Stage 2	Stage 3	Stage 4	Total
Smooth	1390 (92.7%)	106 (7.1%)	4 (0.3%)	0	1500
Irregular	65 (6.6%)	631 (64.5%)	248 (25.4%)	37 (3.8%)	981
Nodular	3 (0.9%)	16 (4.9%)	52 (16.0%)	255 (78.2%)	326
Total	1458	753	304	292	2807

Table 3 Relationship between laparoscopic findings and histological stage in patients with chronic type B hepatitis

Laparoscopic findings	Histological stage				
	Stage 1	Stage 2	Stage 3	Stage 4	Total
Smooth	645 (91.2%)	61 (8.6%)	1 (0.1%)	0	707
Irregular	40 (9.8%)	275 (67.6%)	86 (21.1%)	6 (1.5%)	407
Nodular	4 (1.9%)	20 (9.7%)	62 (30.1%)	120 (58.5%)	206
Total	693	352	159	126	1320

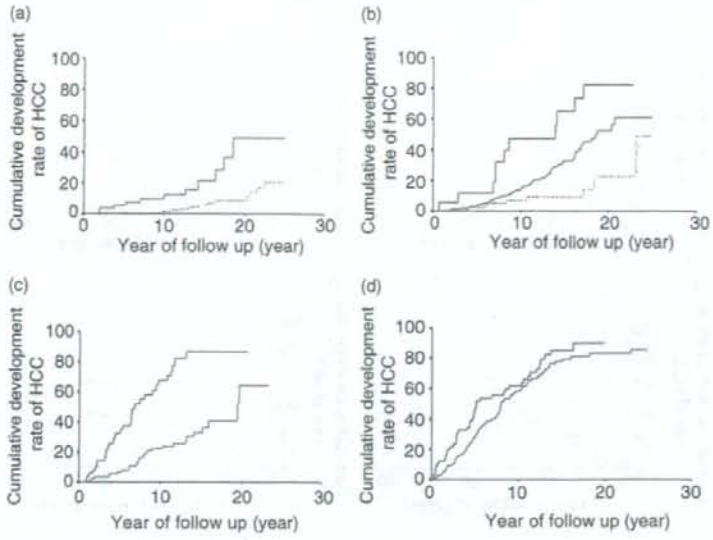

Figure 2 Cumulative development rates of hepatocellular carcinoma (HCC) based on the histological findings of hepatitis C virus patients. (a) Patients with stage 1 ($P < 0.0001$); (b) patients with stage 2 ($P < 0.0001$); (c) patients with stage 3 ($P < 0.0001$, $P = 0.055$); (d) patients with stage 4 ($P = 0.081$). (—) Nodular, (—) Irregular, (—) Smooth.

tional hazard model as shown in Tables 4 and 5. The multivariate Cox regression hazard model using two factors of laparoscopic and histological findings showed that HCC development in the HCV group were independently associated with laparoscopic findings (relative risk based on every progression of one rank [RR], RR = 4.31, $P < 0.0001$) and histological findings (RR = 2.56, $P < 0.0001$). In the HBV group, however, HCC development in was mainly associated with laparoscopic findings (RR = 2.12, $P < 0.0001$) compared to histological finding (RR = 1.13, $P = 0.403$).

The multivariate analysis showed that both laparoscopic and histological findings were associated with HCC appearance in the HCV group. However, in the HBV group, laparoscopic findings were important predictors compared to histological findings.

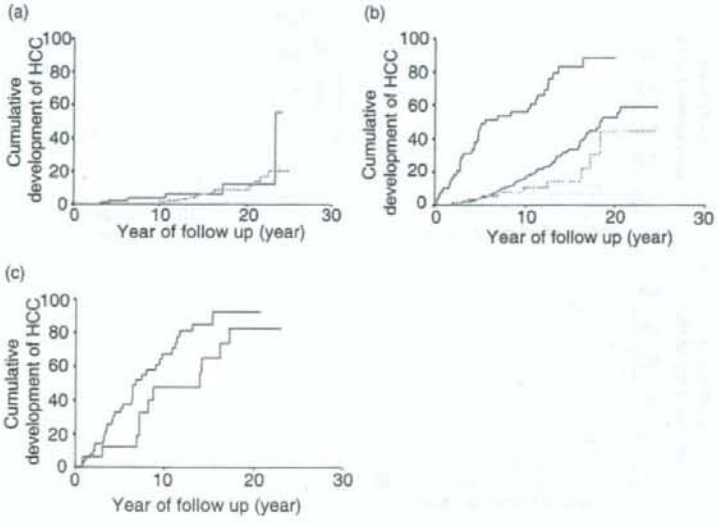

Figure 3 Cumulative development rates of hepatocellular carcinoma (HCC) based on the laparoscopic findings in hepatitis C virus patients. (a) Patients with smooth liver surface ($P < 0.391$); (b) patients with irregular liver surface ($P < 0.0001$); (c) patients with nodular liver surface ($P < 0.055$). (—) Stage 4, ( ) Stage 3, (—) Stage 2, (—) Stage 1.

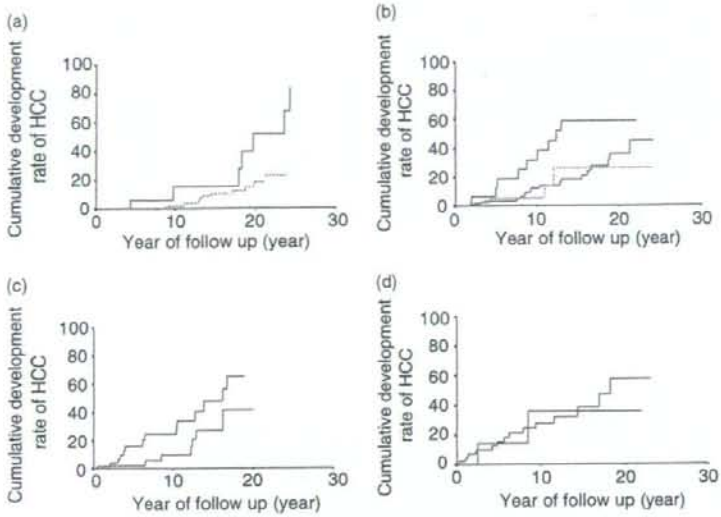

Figure 4 Cumulative development rates of hepatocellular carcinoma (HCC) based on the histological findings in hepatitis B virus patients. (a) Patients with stage 1 ($P < 0.013$); (b) patients with stage 2 ($P < 0.016$); (c) patients with stage 3 ($P < 0.116$); (d) patients with stage 4 ($P < 0.858$). (—) Nodular, (—) Irregular, (—) Smooth.

Adverse event

Seven patients had the following major operative complications: pneumothorax ($n = 6$) and shock due to vasovagal reflux ($n = 1$) at the time of liver biopsy. There were no complications of bleeding at the site of the liver biopsy.

DISCUSSION

WE HAVE DESCRIBED the difference of laparoscopic and histological findings in patients with HCV or HBV. The present study was limited by a retrospective cohort trial. Other limitations of the study were

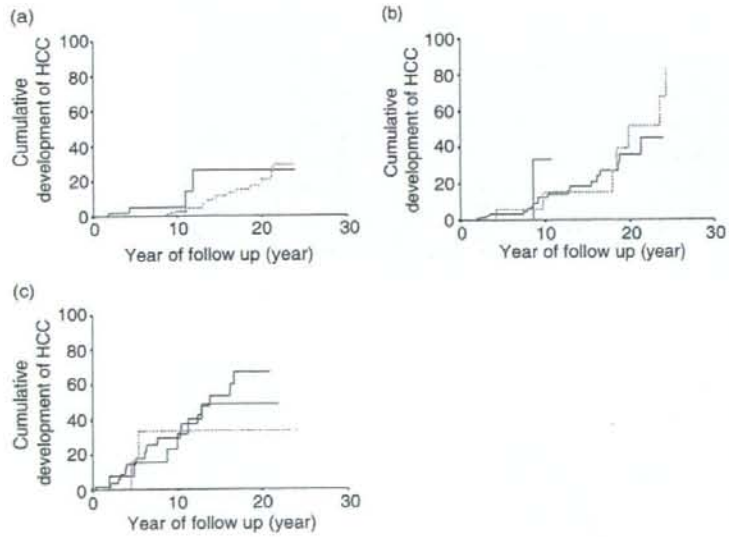

Figure 5 Cumulative development rates of hepatocellular carcinoma (HCC) based on the laparoscopic findings in hepatitis B virus patients. (a) Patients with smooth liver surface ($P < 0.252$); (b) patients with irregular liver surface ($P < 0.736$); (c) patients with nodular liver surface ($P < 0.785$). (—) Stage 4, ( ) Stage 3, (—) Stage 2, (—) Stage 1.

Table 4 Predictive factors for hepatocellular development in hepatitis C virus group

Factor	Category	Risk ratio	95% CI	*P*-value
Univariate analysis				
Histological findings	1: stage 1 2: stage 2 3: stage 3 4: stage 4	2.56	2.34–2.79	<0.0001
Laparoscopic findings	1: smooth 2: irregular 3: nodular	4.31	3.74–4.97	<0.0001
Multivariate analysis				
Histological findings	1: stage 1 2: stage 2 3: stage 3 4: stage 4	1.63	1.39–1.92	<0.0001
Laparoscopic findings	1: smooth 2: irregular 3: nodular	2.31	1.78–2.99	<0.0001

Risk ratio, relative risk based on every progression of one rank in histological and laparoscopic findings. CI, confidence intervals.

that clinical backgrounds of the HCV- and HBsAg-positive patients were significantly different in many factors and the physicians had different experiences on the diagnosis of chronic liver disease. The macroscopic classification by the use of the laparoscopic examination may be subjective, which introduces a further bias; this heterogeneity makes it slightly difficult to interpret the results of the study.

However, the features of the present study are the large study population and prolonged observation study. Moreover, based on irregularities of the liver surface, the laparoscopic findings were classified into only three groups to minimize the subjective bias. The present study shows several findings with regard to laparoscopic and histological examinations in HCV- or HBV-positive patients. First, some patients with a nodular liver surface

Table 5 Predictive factors for hepatocellular development in hepatitis B virus group

Factor	Category	Risk ratio	95% CI	*P*-value
Univariate analysis				
Histological findings	1: stage 1 2: stage 2 3: stage 3 4: stage 4	1.72	1.43–2.07	<0.0001
Laparoscopic findings	1: smooth 2: irregular 3: nodular	2.29	1.79–2.92	<0.0001
Multivariate analysis				
Histological findings	1: stage 1 2: stage 2 3: stage 3 4: stage 4	1.13	0.85–1.49	0.403
Laparoscopic	1: smooth 2: irregular 3: nodular	2.12	1.44–3.14	<0.0001

Risk ratio, relative risk based on every progression of one rank in histological and laparoscopic findings. CI, confidence intervals.

were not diagnosed as having liver cirrhosis when only histological samples were used; in the HBV patients, approximately 45% of patients with a nodular liver surface were not diagnosed as having liver cirrhosis when only histological samples were used. These results suggest that HBV-positive patients with a nodular liver surface tend to have a sampling error compared to HCV-positive patients. A typical liver biopsy represents approximately 1/50 000 of the entire liver surface.[16] Based on this information, we presume that a significant sampling error will be found when diagnosis is based on a single, blind liver biopsy as reported previously.[12–15] Seven patients with a nodular surface were diagnosed as having stage 1, histologically. These seven patients had macro nodular liver surface laparoscopically and showed a part of septa of liver cirrhosis, histologically.

Second, cumulative HCC appearance rates based on the difference of liver surface were more accurate than those based on the difference of the histological findings; this was particularly the case for the HBV patients. In HBV-positive patients with the same histological findings of stage 1, 2, or 3, the HCC development rates differed due to differences in the laparoscopic findings. Using the Cox proportional hazard model, laparoscopic findings were important predictors for HCC development compared to histological findings in the HBV group. On the other hand, both laparoscopic and histological findings were important predictors for HCC development in the HCV group.

Third, there were no complications of bleeding at the biopsy site in our series of 4106 laparoscopies. On the other hand, five of approximately 4100 patients who received US-guided liver biopsy between 1985 and 2000 had bleeding at the biopsy site. The reason for good hemostasis in laparoscopy-guided biopsies is as follows; (i) operators could use gelatin sponge (Gelform) placement to the biopsy site; and (ii) operators could directly check the hemostasis. Laparoscopy offers the advantage of visualization of the liver surface, which leads to greater success in the diagnosis of chronic liver disease than biopsy alone. Although diagnostic laparoscopy is carried out to make an accurate diagnosis of chronic liver disease, the use of laparoscopy accompanies some severe complications. Thus laparoscopy should be examined under the experienced physicians given many training. Moreover, when patients have contraindications, the physician in charge should avoid doing laparoscopy-guided liver biopsy.

The present study suggests that laparoscopic findings are superior to histological findings for predicting HCC development in HBV patients. Based on our results, we recommend that laparoscopy should be considered in the evaluation of chronic liver disease for patients with HBV. When the patients with HBV or HCV are diagnosed without laparoscopic examination, the physician in charge should constantly consider the possibility of cirrhosis and HCC appearance.

In conclusion, our data indicate that laparoscopic findings of the liver are dominant predictors for HCC development compared to histological findings in patients with HBV.

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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,

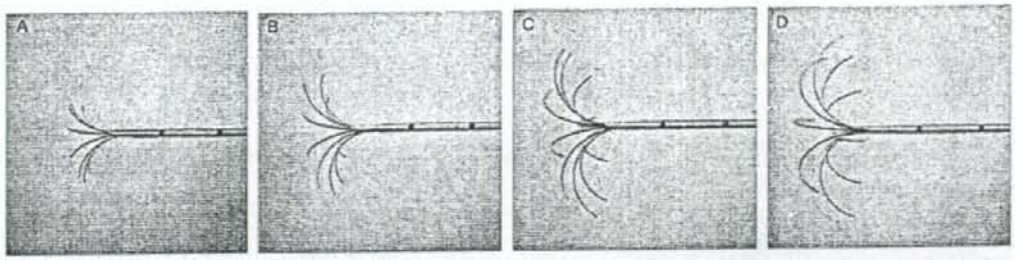

Fig. 1. The conventional four-stepwise procedure. (A) A quarter-length of the electrode tines is expanded in the first step. (B) A half-length is expanded in the second step. (C) A three-quarter length is expanded in the third step. (D) A full-length is expanded in the final step.

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

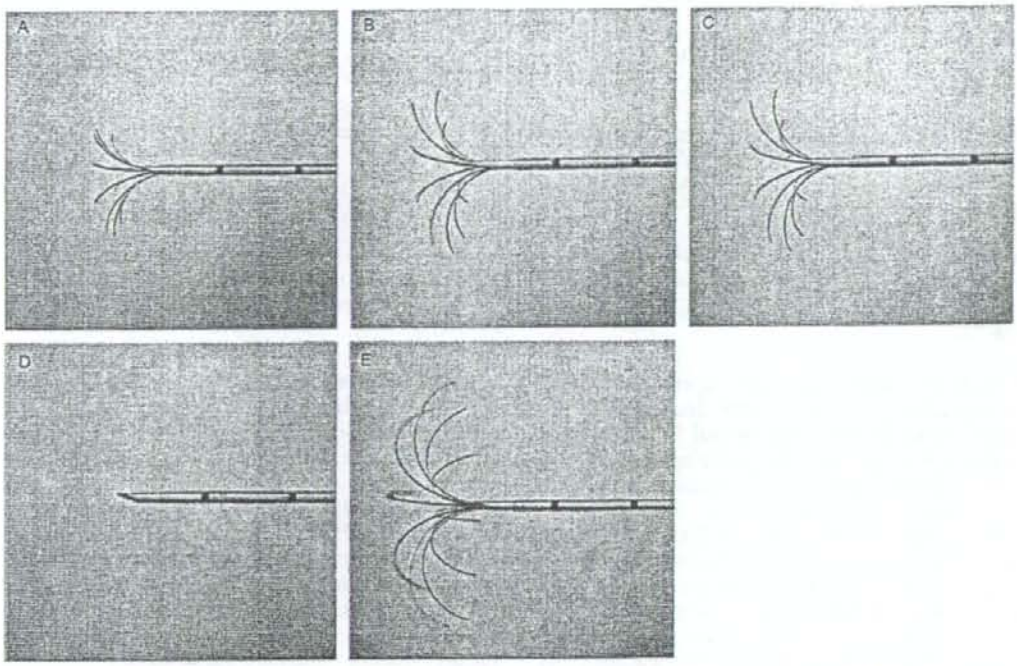

Fig. 2. The new stepwise procedure. (A) A quarter-length of the electrode tines is expanded in the first step. (B) A half-length is expanded in the second step. (C) A three-quarter length is expanded in the third step. (D) The electrode tines are closed in the shaft. (E) Tines are expanded to full length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

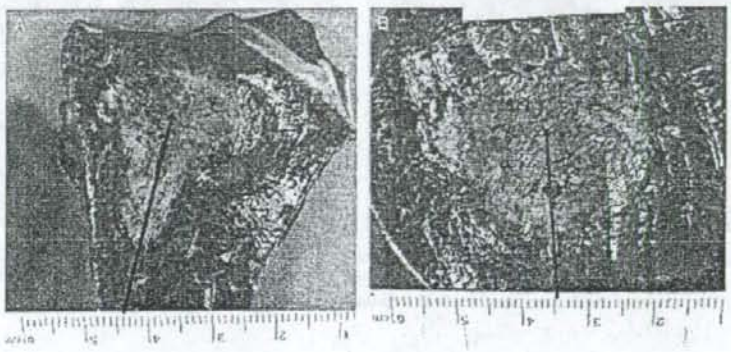

Fig. 3. Photographs of the coagulated area. Arrow shows the direction of the needle shaft. (A) The shape of the area produced by the conventional procedure is cone-like (RFA#3). (B) The shape of the area produced by the new procedure is ellipsoid in shape (RFA#3).

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.

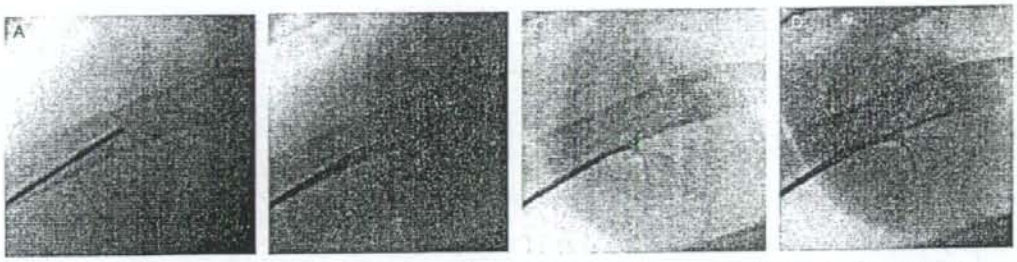

Fig. 4. Electrode tines in a pig liver during the conventional four-stepwise extension procedure. (A) First step. (B) Second step. (C) Third step. (D) Final step. At the second step, the third step and the final step, the progress of the tines' spread is smaller in comparison with that at the first step.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

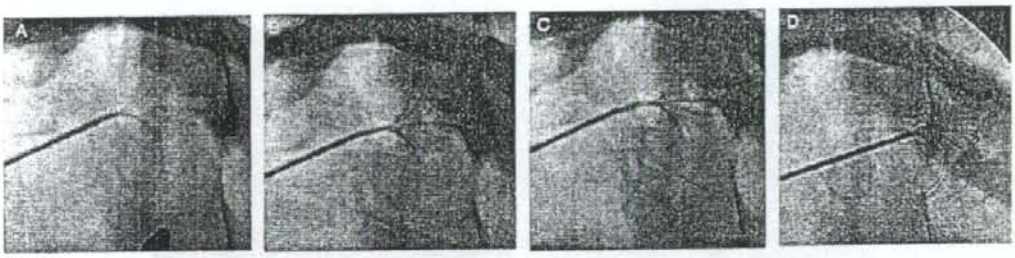

Fig. 5. Electrode tines in a pig liver during the new stepwise extension procedure. (A) First step. (B) Second step. (C) Third step. (D) Final step. At the second step, the third step and the final step, the progress of the tines' spread is smaller in comparison with that at the first step. The extent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

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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.

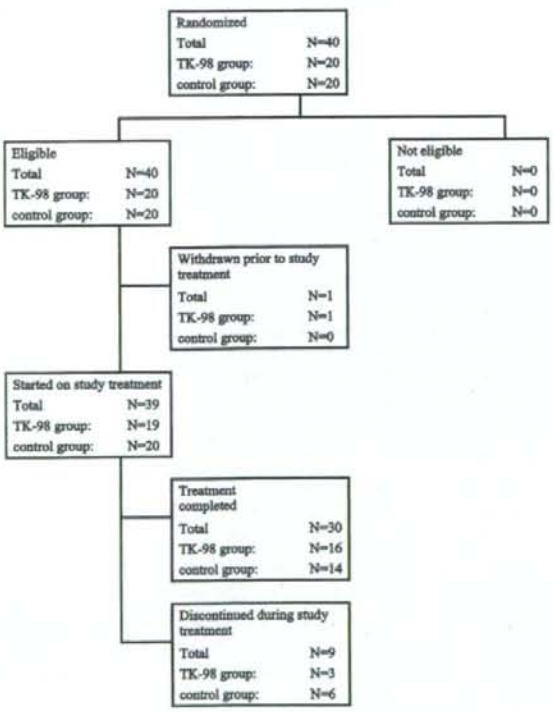

Fig. 1. Flow chart of patients. A total of 39 subjects who initiated study treatment were included in the full analysis set (FAS) and the per protocol set (PPS), as well as a safety analysis

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)

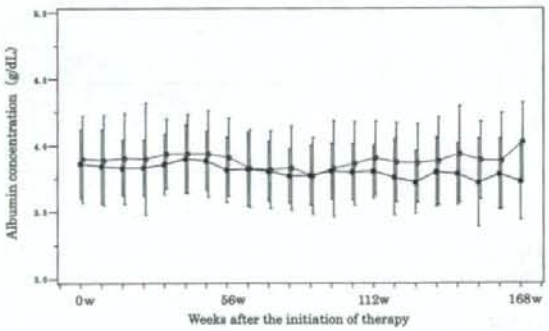

Fig. 2. Serum albumin concentration in TK-98 group (*black dots*) and control group (*white dots*). Data are means; bars show the standard deviation

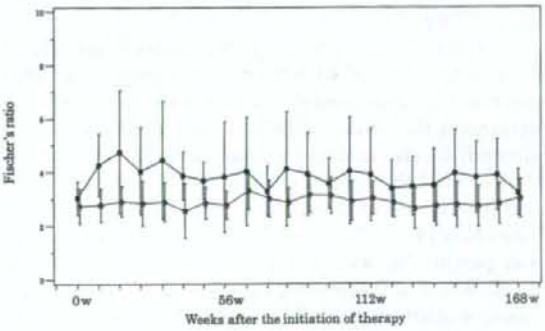

Fig. 3. Fischer's ratio in TK-98 group (*black dots*) and control group (*white dots*). Data are means; bars show the standard deviation

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).

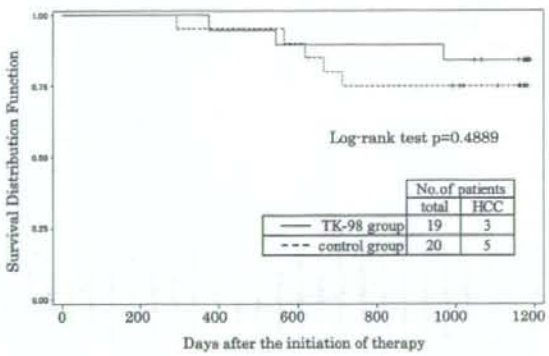

Fig. 4. Kaplan-Meier estimates of event-free survival for hepatocellular carcinoma (*HCC*) in patients with compensated liver cirrhosis caused by hepatitis C virus (HCV) infection

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.

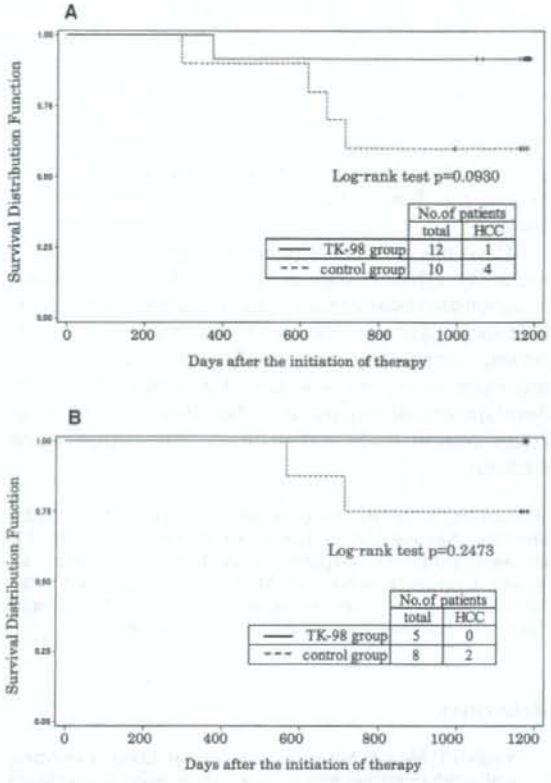

Fig. 5A,B. Kaplan-Meier estimates of event-free survival for HCC in patients with compensated liver cirrhosis caused by HCV infection. **A** Subgroup with a baseline serum albumin level of <4.0 g/dl; **B** subgroup with baseline body mass index (*BMI*) ≥ 25

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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