Sciences (NUGSBS) between 2007 and July 2010. In this study, all patients were examined with the Sonazoid IOUS since 2007 after its approval by the Japanese health authorities. The liver tumors included HCC in 25 patients, intrahepatic cholangiocarcinoma (ICC) in 3, colorectal liver metastasis in 20, liver metastasis of gastrointestinal stromal tumor from the stomach in one and benign hematoma (preoperative diagnosis was HCC) in one. The patients included 34 males and 16 females, with a mean age at the time of surgery of 68.1 ± 11.1 years (±SD, range, 36–84 years). The background liver disease included normal liver in 22 patients, alcoholic liver dysfunction in two, chronic viral hepatitis in 15, and cirrhosis due to viral hepatitis in 11. The operative procedures included hemihepatectomy in 8 patients, segmentectomy or sectionectomy in 18, and partial resection in 24 (48%). Radio-frequency ablation had been performed in two patients with small HCC measuring <1 cm in diameter. Radical hepatectomy was performed and hepatic tumors were completely resected without macroscopic exposure of the amputated section to the remaining liver.

As a control group, data of 81 patients, including those with HCC in 40, liver metastasis in 32, and ICC in 9, who underwent hepatectomy under conventional IOUS between 2004 and 2006, were used for comparison to those of the present series. The control group included 62 males and 19 females, with a mean age at the time of surgery of 65.2 ± 12.7 years (range, 27–85 years). The operative procedures included hemihepatectomy in 16 patients, segmentectomy or sectionectomy in 17, and partial resection in 48 (59%). Comparative study between Sonazoid IOUS group and the conventional group was historically attempted. All study protocols were approved by the Human Ethics Review Board of our institution. Informed consent for data collection was obtained from each patient during this period. Anesthesia data and patient data were retrieved from the NUGSBS database.

Procedure of IOUS and Administration of Sonazoid Agent

Examination of IOUS and the contrast-enhanced IOUS was performed using an Xario™ XG (Toshiba Medical Systems, Tokyo, Japan) and a micro-convex probe (PVT-375BT, 3.5 MHz, Toshiba Medical Systems) and a interoperative probe (PLT-705BTH, 7.5 MHz). All intraoperative IOUS were performed by surgeons to help in the decision on the indication for hepatectomy. At first, we examined the two-dimensional (2D) images of the tumor and its location, and also Color Doppler Images of the tumor vasculature [12]. We separately scanned the shallow area and the deep or bottom area of the liver by changing the intensity of the echo according to the depth of the tumor. For tumors located on the liver surface, the liver was covered with warm saline to reduce the air-gap between the probe and the liver surface. An intravenous bolus injection of 0.5 ml of perflubutane microbubble (Sonazoid) was administrated via the peripheral vein at the time of laparotomy incision to observe the Kupffer-phase image at 1–2 hr after injection because the microbubbles remain for a long time [12]. On the micro-flow imaging, the contrast harmonic imaging of the liver and tumor was observed. Dual monitor real-time view of the conventional 2D image and micro-flow images of Sonazoid–IOUS could be observed. First, the late Kupffer-phase image was observed under low value of mechanical index (MI; an index of mechanical impact of the ultrasonic waves on the target) of 0.3 and scanning of the liver tumor was performed to ensure the integrity of the Sonazoid microbubble structure, because Sonazoid microbubbles can burst at high MI (>0.3). The liver tumor was identified as a low contrast perfusion defect compared with the normal liver parenchyma (Fig. 1a,b). Using this image, we examined the tumor morphology, surrounding daughter nodules, vascular infiltration, scanning of any tumor lesions that could not be identified by preoperative imaging, and

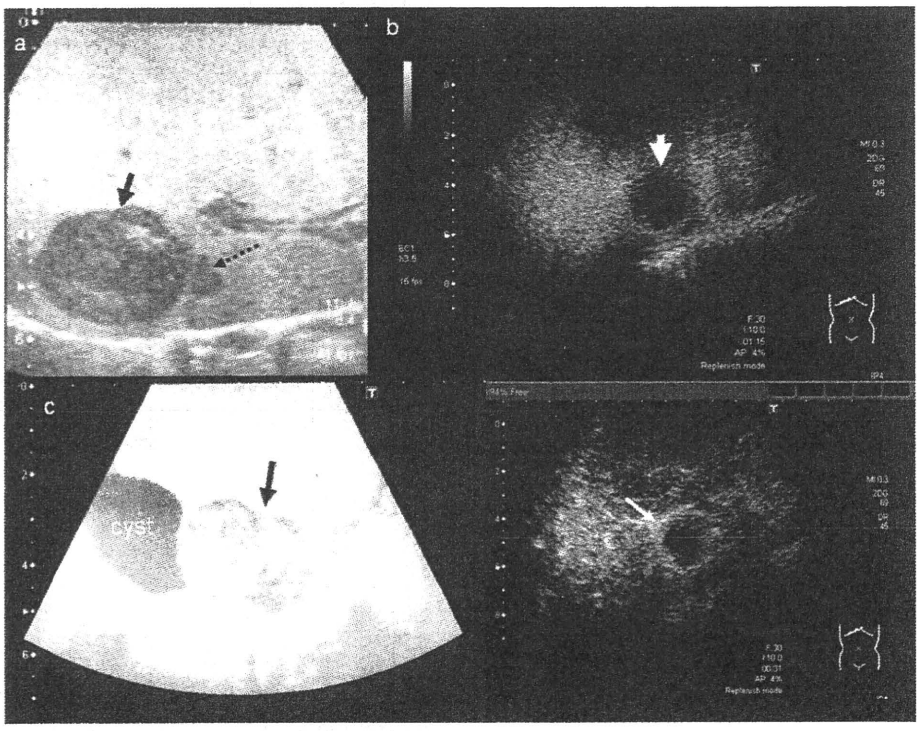

Fig. 1. a: Representative late Kupffer-phase image of HCC (arrow) with extra-capsular tumor growth (dotted arrow). b: Kupffer image of colorectal liver metastasis (white arrow). At the early arterial phase, the HCC image showed hypervascularity in the tumor (c) and vascularity around the colorectal liver metastasis (d).

set-up of surgical margin. In the next step, another bolus dose of Sonazoid was injected to examine the characteristics of the vascular flow of intra- or extra-tumor lesions at an early vascular phase within 3 min after the injection (Fig. 1c,d) [16]. If necessary, vascular imaging by reperfusion of the tumor after destruction of the microbubbles using high MI (>1.0) was examined to confirm vascular flow at the arterial phase [10].

Statistical Analysis

All continuous data were expressed as mean ± SD. Data of different groups were compared using one-way analysis of variance (ANOVA). Chi-square test was used for comparison of categorical variables. A two-tailed *P*-value <0.05 was considered significant. StatView Software for Windows, version 5.0 (SAS Institute, Inc., Cary, NC) was used for all statistical analyses.

RESULTS

Diagnosis of Liver Tumors by Sonazoid

Table I shows the procedure records of Sonazoid-enhanced IOUS. Scanning the whole liver by Sonazoid–IOUS was accomplished in all patients. Sonazoid injection was performed twice in many cases. No side effects for Sonazoid injection were registered in the present series.

The mean tumor size was 3.4 ± 1.9 cm (range, 0.8–6.2 cm). Table II shows the image findings of Sonazoid–IOUS. We started viewing the late Kupffer-phase images and the main liver tumors were clearly detected as perfusion defects in most cases except for one case with HCC (well-to-moderately differentiated HCC), which showed an ill-defined low echoic area. Although intratumor revascularization at the arterial phase was not clearly confirmed, the poor vascularization was confirmed by the reperfusion technique. Vascularization of intra- and extra-tumorous lesion at the arterial or early portal phase within 15 sec was observed in 92%. Although extra-tumorous enhancement of colorectal liver metastasis, intrahepatic cholangiocellular carcinoma, and intratumorous vascularization of GIST was observed in all patients, intratumorous vascularization was not clearly detected in one case as described above. In HCC, a small size lesion (7 mm) was clearly detected in one, extra-capsular tumor growth of the main tumor was clearly detected in one case (Fig. 1a), portal vein tumor thrombus (PVTT) was clearly detected in three (Fig. 2a), and the distance between HCC and the main hepatic vein was confirmed in five. In colorectal liver metastasis, infiltration adjacent the Glissonian branch was detected in one case (Fig. 2b). The preoperative diagnosis in one case based on enhanced CT and MRI findings was HCC with hypervascularity; however, the pathological finding showed hematoma of unknown etiology. Sonazoid–IOUS showed no vascularization in the tumor region or surrounding tissue although the Kupffer-phase images showed a perfusion defect.

New small liver tumors were observed by Sonazoid–IOUS in three cases (Table II). One 8-mm HCC was detected in segment four in one case, which was ablated by radio-frequency coagulation intraoperatively. Two colorectal metastases measuring 3 and 5 mm in size were detected, which were resected and pathologically diagnosed as liver metastasis in both cases. In three cases, treatment of liver tumors was applied based on equivocal diagnosis of malignancy by CT or MRI preoperatively. Radio-frequency ablation was performed in two cases and transarterial chemoembolization was performed in one. The late Kupffer-phase images showed complete perfusion defect but no vascularization was observed following reinjection or reperfusion technique in these lesions. Eventually, neither intraoperative biopsy nor treatment could be performed because of the small size of the tumor but tumor relapse was not detected in these lesions after surgery (the mean follow-up period was 8.6 ± 4.2 months). We also found

TABLE I. Records of Contrast-Enhanced Intraoperative Ultrasonography Using Sonazoid

Amount of Sonazoid	Number of cases
0.5 ml × twice	47
0.5 ml × 3 times	3

Adverse effects: nil.

TABLE II. Findings of Main Liver Tumors and Accessory Lesions by Sonazoid–IOUS

Main tumors	49/50 (98%)
Clearly visible at the late Kupffer phase	
Clearly visible at early Kupffer phase	48/50 (96%)
HCC (intratumor vascularity)	23/25 (92%)
Metastatic GIST (intratumor vascularity)	1/1
CCC (surrounding area of tumor)	3/3
Colorectal metastasis	20/20 (100%)
Hematoma	0/1
Accessory lesions	
Detection of new malignant lesions	5
Detection of pretreated lesions	3
Suspicious lesions of malignancies	5
Differential diagnosis of benign lesions	10
Small (<1 cm) or multiple cysts	6
Small (<1 cm) hemangioma	3

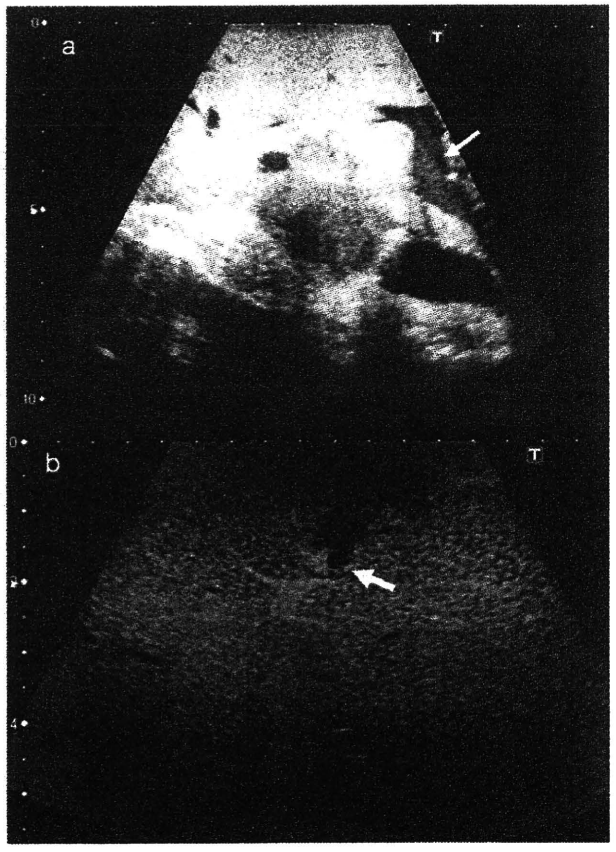

Fig. 2. Findings of vascular involvements. **a**: Portal vein thrombus invasion from HCC (white arrow). **b**: Colorectal liver metastasis invading the adjacent portal vein (arrow).

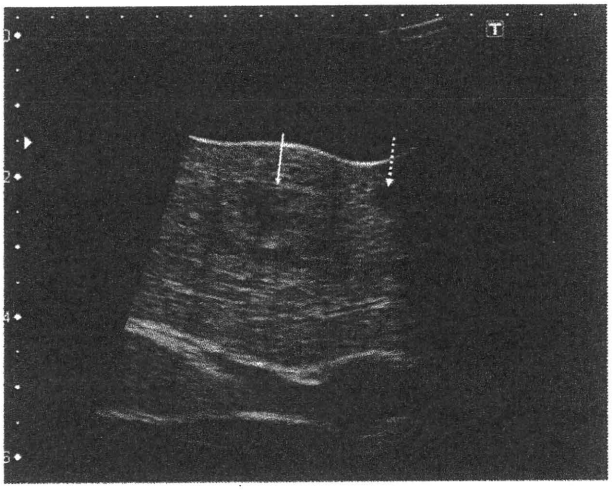

Fig. 3. Neighboring cyst of colorectal liver metastasis was clearly observed on the enhanced images.

suspicious lesions as tumor lesions in five cases. They comprised 3–5 mm lesions in case of colorectal metastasis and no perfusion defect images were observed in the late Kupffer phase in three cases. Furthermore, two other 5–7 mm lesions were suspected as HCC by CT in two cases; however, no early enhancement or perfusion defect at late phase was observed. Intraoperative biopsy or treatment was not performed but no tumor relapse was noted in these lesions after surgery. The differential diagnosis of benign lesions was made in 10 cases including liver cysts in 7 cases and hemangioma in 3. In a case of liver cyst, the preoperative diagnosis based on MRI was liver metastasis (Fig. 3). These lesions had typical findings of cysts with remarkable acoustic back echo and contrast to the liver parenchyma, while that of hemangioma as a cotton–wool like pooling image. No biopsy or treatment was performed for these lesions.

Surgical Results Using Sonazoid–IOUS

We compared the presence of carcinoma at the surgical margin in patients with liver tumors who underwent hepatectomy under conventional IOUS (Table III). The mean tumor size was 4.2 ± 2.5 cm (range, 1.0–6.2 cm), which was not significantly different from that in the Sonazoid–IOUS group ($P = 0.18$). The proportion of surgical margin-positive cases using the Sonazoid–IOUS (8%) was not significantly different from that using the conventional IOUS (17%, $P = 0.39$). In colorectal liver metastasis and ICC, the proportion of cases with cancer-positive margin was similar in the two groups (17% vs. 20%; $P = 1.0$). In HCC, the proportion of cases with cancer-positive margin tended to be lower in the Sonazoid IOUS group than in the conventional IOUS group (0% vs. 15%) but the difference was not significant ($P = 0.073$).

TABLE III. Detection of Cancer in Surgical Margin by Sonazoid–IOUS and Conventional IOUS

	Cancer-positive	Cancer-negative	*P*-value
All cases			
Sonazoid IOUS	4	45	0.19
Conventional IOUS	14	67	
HCC			
Sonazoid IOUS	0	25	0.073
Conventional IOUS	6	34	
CLM and ICC			
Sonazoid IOUS	4	20	1.0
Conventional IOUS	8	33	

IOUS, intraoperative ultrasonography; HCC, hepatocellular carcinoma; CLM, colorectal liver metastasis; ICC, intrahepatic cholangiocarcinoma.

DISCUSSION

In 2007, a new contrast media of microbubble agent, Sonazoid was approved for use in Japan for the diagnosis of liver tumors including HCC, CCC, and liver metastasis [12,13,17–20]. The introduction of this modality markedly improved the diagnostic accuracy of liver tumors, to levels similar to those of gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA)-MRI [10,13,17]. The use of Sonazoid–IOUS has allowed the detection of occult lesions undetected by the conventional US [9,12,19,20]. Particularly, the Kupffer-phase imaging is a powerful diagnostic tool to identify even small size abnormal nodules [9]. Following confirmation of the usefulness of contrast Sonazoid–IOUS, this modality has been applied in patients undergoing percutaneous ablation therapy or for evaluation of treatment-scheduled lesions by RFA or TACE [16,21,22]. The usefulness of IOUS has been well realized and IOUS is considered a reliable imaging technique often used to confirm the diagnosis of intrahepatic tumor lesions [5,6]. In the present study, we expected that contrast IOUS using Sonazoid would enhance the detection of lesions based on defining tumor vascularity, location, and decision of sufficient surgical margin. The advantages of contrast IOUS using Sonazoid were recently reported in patients with colorectal liver metastasis [12,14,15]. However, the significance of IOUS using Sonazoid has not been investigated and, to our knowledge, its application for HCC and other liver tumors has not been reported. In the present study, we assessed the usefulness of Sonazoid IOUS in the detection of various liver tumors as a preliminary study. Sonazoid injection was safe and not associated with any side effects, as reported previously [23].

Using the same Sonazoid–IOUS protocol described in previous studies [17,24], we examined the early arterial phase first followed by vascularity at the portal phase or perfusion defect images at the late Kupffer phase [17,24]. However, the procedure is time consuming as it takes more than 30 min for full examination, and thus needs to be shortened. The Sonazoid–IOUS allows searching the entire liver for tumors over a relatively long period by screening for perfusion defect on the Kupffer-phase images because defect on the images lasts for a long time due to the stability of the Sonazoid agent [23]. Therefore, we first examined perfusion defect images after injection of the contrast medium at laparotomy before any surgical decision is made. Subsequently, Sonazoid is reinjected or reperfused to examine only the targeted lesions. Based on this modification, we were able to shorten the time of examination. In the present series, all liver tumors could be detected by Sonazoid–IOUS. Of the 50 patients, we attempted preoperative Sonazoid–US in 2 patients who had suspicious small lesions in segment 8 on magnetic resonance images. However, these were not detected by preoperative US. In contrast, these lesions could be detected by Sonazoid–IOUS. Small liver tumors at segment 7 or 8 are difficult to visualize by extra-corporeal Sonazoid–US because of lung echogenicity. Therefore, IOUS could overcome the limitation of the conventional US [12,16], making it a powerful diagnostic tool. In one case of well-differentiated HCC, no clear perfusion defect could be observed. In this regard, Korenaga et al. [17] reported that the Kupffer-phase images are excellent in moderately or poorly differentiated HCC [17]. In another case of hematoma that was diagnosed preoperatively as HCC by CT and MRI, no lesion was detected on early vascularization images of Sonazoid IOUS, suggesting that this modality may be more sensitive for differential diagnosis [9,10,13,17,22,25]. Joshita et al. [18] reported the echoic pattern of ICC by Sonazoid–US, which showed hypoechoic finding and unclear margin at Kupffer phase. In our series, ICC was observed in two cases,

which also showed hypovascularity similar to liver metastasis. ICC sometimes showed hypervascularity on contrast CT as described previously by our group and others [26,27]. Thus, further studies of a larger series of patients are necessary to clarify the echogenicity of ICC.

Occult metastatic lesions, which were not detected by preoperative examinations and IOUS, were detected in 19–25% and additional resection was necessary as reported previously [12,14,15]. In the present study, occult metastatic lesions (i.e., a new small tumor) were detected in 5 of 50 cases using Sonazoid–IOUS and were later removed by additional hepatectomy. These metastatic lesions were not detected by preoperative CT, MRI, or the conventional IOUS, similar to previous reports [12,28]. In the present series, the new small lesions were observed near the portal veins. If these lesions were not detected, they could have invaded the vessels following tumor growth. In HCC, PVTT is a typical pattern of tumor invasion [29] and it is important to detect and resect lesions infiltrating the Glissonian pedicles for curable resection. The present case showed a clear PVTT, with clear vascularity in the thrombus. The contrast with the portal flow was very clear and it is easy to detect the extent of PVTT or margin. As this finding is sometimes difficult to detect by conventional US, Sonazoid US seems to be more useful for the detection of intravascular tumor thrombus or vascular infiltration of liver malignancies.

In patients who undergo hepatectomy, some patients occasionally undergo ablation therapy or TACE prior to operation. This results in alteration of the echogenicity of the treated area, appearing as hypoechoic lesions [21,22,30]. It may be difficult to accurately differentiate between necrotic lesions and viable tumors by the conventional US. However, early vascularization in the tumor area or surrounding region is a sign of tumor viability [31,32] and Sonazoid US might be useful for the differential diagnosis of tumors or suspicious lesions in the treated areas.

Luo et al. [19] reported the usefulness of Sonazoid–US for the diagnosis of both benign and malignant liver tumors. Liver cyst or hemangioma is a common benign lesion in the liver, and shows typical ultrasonic findings [19,20,33]. However, differentiating small size lesion like these from liver carcinomas is often difficult [33]. In the present study, Sonazoid–IOUS showed a clear contrast between such a lesion and the liver parenchyma, suggesting that it might be useful for the detection of such lesions. Vascular enhancement of hemangioma is typical, which could be easily detected by this modality as well [34]. With regard to other benign liver lesions such as focal nodular hyperplasia (FNH) or liver adenoma resembling HCC, differential diagnosis using the Sonazoid–IOUS modality is promising to help in the clinical decision making regarding the selection of operative technique during surgery. Although we have no extensive experience regarding such lesions, Luo et al. [35] reported that the sensitivity of diagnosis of FNH by the contrast US was as high as 80%.

Eventually, we aim to increase complete resection with negative surgical margin of carcinomas using the Sonazoid–IOUS. Although the Sonazoid–IOUS was compared with the conventional IOUS in the present study, no significant differences were observed. However, in HCC, a cancer-positive margin was not observed in the Sonazoid–IOUS group. As prevalence of cancer-positive margin tended to be lower relative to the conventional IOUS (but not statistically significant), this modality might be useful to define surgical margin based on the Kupffer-phase images. We applied Sonazoid–IOUS during hepatic resection to observe the surgical margin in the present series, and the results were not different from the conventional technique [5,6]. In case of limited hepatectomy, the surgical margin may become smaller in comparison with major hepatectomy. The incidence of partial resection tended to be higher in the control group than the Sonazoid–IOUS group though no significant difference was detected in the background between the two groups. As described above, a small or unclear infiltration of the Glissonian branches can be detected by Sonazoid–IOUS and, therefore, it is helpful to reduce the remnant tumor thrombus in the adjacent vessels in patients with HCC [29], which could also lead to a negative surgical margin. Sonazoid–IOUS may help provide better surgical curability in comparison with the conventional IOUS. Our concern was difficulty in detecting tumor lesions simultaneously present on the liver surface and back since the focus on the layer of interest is necessary. It is necessary to change the MI value according to the depth of the lesion. It is still difficult to detect tumors on the liver surface of patients with cirrhotic liver. In the present series, we immersed the liver in saline to find such lesions. Based on this preliminary study, usefulness of Sonazoid–IOUS was estimated and, therefore, the further study with a large number of patients will be necessary in the next step.

CONCLUSION

In conclusion, we have demonstrated that contrast media IOUS using the new microbubble agent, Sonazoid, allows easy examination of the location and extent of liver tumors in the whole liver and to detect occult tumor lesions and assist in decision making of hepatic resection. This relatively new imaging modality could become a standard procedure in patients with liver tumors who undergo hepatectomy.

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REFERENCES

1. Song TJ, Ip EW, Fong Y: Hepatocellular carcinoma: Current surgical management. Gastroenterology 2004;127:S248–S260.
2. Mayo SC, Pawlik TM: Current management of colorectal hepatic metastasis. Expert Rev Gastroenterol Hepatol 2009;3:131–144.
3. Guglielmi A, Ruzzenente A, Campagnaro T, et al.: Intrahepatic cholangiocarcinoma: Prognostic factors after surgical resection. World J Surg 2009;33:1247–1254.
4. Altendorf-Hofmann A, Scheele J: A critical review of the major indicators of prognosis after resection of hepatic metastases from colorectal carcinoma. Surg Oncol Clin N Am 2003;12:165–192.
5. Torzilli G, Makuuchi M: Intraoperative ultrasonography in liver cancer. Surg Oncol Clin N Am 2003;12:91–103.
6. Poon RT: Recent advances in techniques of liver resection. Surg Technol Int 2004;13:71–77.
7. Calliada F, Campani R, Bottinelli O, et al.: Ultrasound contrast agents: Basic principles. Eur J Radiol 1998;27:S157–S160.
8. Sontum PC: Physicochemical characteristics of Sonazoid, a new contrast agent for ultrasound imaging. Ultrasound Med Biol 2008; 34:824–833.
9. Numata K, Morimoto M, Ogura T, et al.: Ablation therapy guided by contrast-enhanced sonography with Sonazoid for hepatocellular carcinoma lesions not detected by conventional sonography. J Ultrasound Med 2008;27:395–406.
10. Kudo M: Multistep human hepatocarcinogenesis: Correlation of imaging with pathology. J Gastroenterol 2009;44:112–118.
11. Yanagisawa K, Moriyasu F, Miyahara T, et al.: Phagocytosis of ultrasound contrast agent microbubbles by Kupffer cells. Ultrasound Med Biol 2007;33:318–325.
12. Nakano H, Ishida Y, Hatakeyama T, et al.: Contrast-enhanced intraoperative ultrasonography equipped with late Kupffer-phase image obtained by sonazoid in patients with colorectal liver metastases. World J Gastroenterol 2008;14:3207–3211.
13. Inoue T, Kudo M, Hatanaka K, et al.: Imaging of hepatocellular carcinoma: Qualitative and quantitative analysis of postvascular phase contrast-enhanced ultrasonography with sonazoid.

Comparison with superparamagnetic iron oxide magnetic resonance images. Oncology 2008;75:48–54.
14. Torzilli G, Del Fabbro D, Palmisano A, et al.: Contrast-enhanced intraoperative ultrasonography during hepatectomies for colorectal cancer liver metastases. J Gastrointest Surg 2005;9:1148–1154.
15. Leen E, Ceccotti P, Moug SJ, et al.: Potential value of contrast-enhanced intraoperative ultrasonography during partial hepatectomy for metastases: An essential investigation before resection? Ann Surg 2006;243:236–240.
16. Miyamoto N, Hiramatsu K, Tsuchiya K, et al.: Sonazoid-enhanced sonography for guiding radiofrequency ablation for hepatocellular carcinoma: Better tumor visualization by Kupffer-phase imaging and vascular-phase imaging after reinjection. Jpn J Radiol 2009;27:185–193.
17. Korenaga K, Korenaga M, Furukawa M, et al.: Usefulness of Sonazoid contrast-enhanced ultrasonography for hepatocellular carcinoma: Comparison with pathological diagnosis and superparamagnetic iron oxide magnetic resonance images. J Gastroenterol 2009;44:733–741.
18. Joshita S, Ichijo T, Suzuki F, et al.: A case of well-differentiated cholangiolocellular carcinoma visualized with contrast-enhanced ultrasonography using Sonazoid. Hepatol Res 2009;39:207–212.
19. Luo W, Numata K, Kondo M, et al.: Sonazoid-enhanced ultrasonography for evaluation of the enhancement patterns of focal liver tumors in the late phase by intermittent imaging with a high mechanical index. J Ultrasound Med 2009;28:439–448.
20. Wen YL, Kudo M, Zheng RQ, et al.: Characterization of hepatic tumors: Value of contrast-enhanced coded phase-inversion harmonic angio. AJR Am J Roentgenol 2004;182:1019–1026.
21. Luo W, Numata K, Morimoto M, et al.: Role of Sonazoid-enhanced three-dimensional ultrasonography in the evaluation of percutaneous radiofrequency ablation of hepatocellular carcinoma. Eur J Radiol 2010;75:91–97.
22. Xia Y, Kudo M, Minami Y, et al.: Response evaluation of transcatheter arterial chemoembolization in hepatocellular carcinomas: The usefulness of sonazoid-enhanced harmonic sonography. Oncology 2008;75:99–105.
23. Landmark KE, Johansen PW, Johnson JA, et al.: Pharmacokinetics of perfluorobutane following intravenous bolus injection and continuous infusion of sonazoid in healthy volunteers and in patients with reduced pulmonary diffusing capacity. Ultrasound Med Biol 2008;34:494–501.
24. Maruyama H, Takahashi M, Ishibashi H, et al.: Ultrasound-guided treatments under low acoustic power contrast harmonic imaging for hepatocellular carcinomas undetected by B-mode ultrasonography. Liver Int 2009;29:708–714.
25. Shiozawa K, Watanabe M, Sumino Y: Evaluation of the hemodynamic status of focal hepatic lesions 20 mm or less in diameter by contrast-enhanced ultrasonography using Sonazoid. Intervirology 2009;52:213–222.
26. Nanashima A, Sumida Y, Abo T, et al.: Relationship between pattern of tumor enhancement and clinicopathologic characteristics in intrahepatic cholangiocarcinoma. J Surg Oncol 2008;98:535–539.
27. Yamamoto M, Ariizumi S, Otsubo T, et al.: Intrahepatic cholangiocarcinoma diagnosed preoperatively as hepatocellular carcinoma. J Surg Oncol 2004;87:80–83.
28. Harvey CJ, Lim AK, Blomley MJ, et al.: Detection of an occult hepatocellular carcinoma using ultrasound with liver-specific microbubbles. Eur Radiol 2002;12:S70–S73.
29. Minagawa M, Makuuchi M: Treatment of hepatocellular carcinoma accompanied by portal vein tumor thrombus. World J Gastroenterol 2006;12:7561–7567.
30. Owen NR, Chapelon JY, Bouchoux G, et al.: Dual-mode transducers for ultrasound imaging and thermal therapy. Ultrasonics 2010;50:216–220.
31. Hotta N, Tagaya T, Maeno T, et al.: Advanced dynamic flow imaging with contrast-enhanced ultrasonography for the evaluation of tumor vascularity in liver tumors. Clin Imaging 2005;29:34–41.
32. Shimizu M, Iijima H, Horibe T, et al.: Usefulness of contrast-enhanced ultrasonography with a new contrast mode, Agent Detection Imaging, in evaluating therapeutic response in hepatocellular carcinoma treated with radio-frequency ablation therapy. Hepatol Res 2004;29:235–242.
33. Nanashima A, Takeshita H, Sawai T, et al.: Preoperative assessment of liver metastasis originating from colorectal carcinoma: Is super paramagnetic iron oxide particles-magnetic resonance imaging (SPIO-MRI) useful for screening? Hepatogastroenterology 2008;55:1750–1753.
34. Wang WP, Wu Y, Luo Y, et al.: Clinical value of contrast-enhanced ultrasonography in the characterization of focal liver lesions: A prospective multicenter trial. Hepatobiliary Pancreat Dis Int 2009;8:370–376.
35. Luo W, Numata K, Morimoto M, et al.: Three-dimensional contrast-enhanced sonography of vascular patterns of focal liver tumors: Pilot study of visualization methods. AJR Am J Roentgenol 2009;192:165–173.

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Original Article

Effect of an oral branched chain amino acid-enriched snack in cirrhotic patients with sleep disturbance

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Aim: Sleep is closely related to physical and mental health. Sleep disturbance is reported in patients without encephalopathy. We examined the relationship among cirrhotic symptoms, laboratory data and sleep disturbances. Next, we examined the influence of a branched chain amino acid (BCAA) supplement on sleep disturbance in cirrhotic patients.

Methods: We investigated a total of 21 patients at Nagasaki University Hospital from January to June 2009. We constructed questionnaire items for the evaluation of cirrhotic symptoms. The items, as major symptoms of cirrhotic patients, were as follows: hand tremor, appetite loss, muscle cramp of foot, fatigue, decreased strength, anxiety, abdominal fullness, abdominal pain and a feeling of low energy. We used the Epworth Sleepiness Scale (ESS) for the evaluation of daytime hypersomnolence. Energy supplementation with a BCAA snack was performed as a late evening snack (LES). All patients were assessed at the time of entry into the study, and at 4 and 8 weeks.

Results: It was found that BCAA snack, taken p.o. as an LES, improved the ESS for cirrhotic patients without encephalopathy. This beneficial result was recognized in the short term, 4 weeks after beginning of treatment. This study demonstrated the utility of BCAA supplementation for cirrhotic patients with sleep disturbance. However, the cirrhotic symptom-related score was positively relation with the Child–Pugh score at the time of patient entry, and we were unable to identify the item that related to ESS.

Conclusion: A BCAA snack is a useful drug for cirrhotic patients who do not have any overt encephalopathy, but who suffered from sleep disturbance.

Key words: branched chain amino acid supplement, Epworth Sleepiness Scale, liver cirrhosis, late evening snack.

INTRODUCTION

THE PATIENTS WITH liver cirrhosis (LC) have a wide range of symptoms. A few cirrhotic patients have sleep disturbance.[1] In recent years, it has been reported that sleep is closely related to physical and mental health.[2,3] Sleep disturbance is one of the symptoms of overt hepatic encephalopathy,[4] but has been reported in patients without encephalopathy.[1] In a previous report,[1] a questionnaire indicated an elevated number (47.7%) of cirrhotic patients who complained of unsatisfactory sleep compared with healthy control subjects (4.5%). Additionally, global sleep quality was significantly lower in the primary biliary cirrhosis group[5] and the non-alcoholic fatty liver disease group[6] compared to control subjects. In Japan, the overall prevalence of insomnia during the preceding month was found to be 21.4% among the general Japanese population.[7] In the present study, we examined the circumstances of the sleep disturbances in Japanese cirrhotic patients.

The relationship between sleep disturbance and variegated cirrhotic symptoms are still not clear. Previous reports indicated that sleep disturbances are not related to liver function.[1,8] It has been reported that fatigue in non-alcoholic fatty liver disease patients is significant, and is associated with excessive daytime sleepiness but not with insulin resistance.[6] Because of the emergence of sleep disturbances in cirrhotic patients without encephalopathy, the relationship between sleep disturbances and cirrhotic symptoms, excluding encephalopathy, should be examined. In this study, we constructed

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an original symptom evaluation, and examined the relationship between sleep disturbance and other cirrhotic symptoms.

The energy balance of LC was characterized as protein-energy malnutrition (PEM), involved disorder of glycolysis, decline of glycogenosis, negative nitrogen balance and hyper-lipolysis.[9–11] PEM carries a high risk of morbidity and mortality by increasing the risk of life-threatening complications, which in turn reduce quality of life (QOL) independently of liver function.[12,13] Recently, branched-chain amino acids (BCAA) supplementation in patients with liver disease has gained attention. The administration of BCAA has been shown to correct malnutrition associated with LC in both animal and human studies.[14,15] Additionally, it has been reported that long-term nutritional BCAA supplementation is useful to prevent prognostic hepatic failure and to improve surrogate markers in advanced LC.[15–17] It has been described that BCAA supplementation is effective in downregulating protein metabolism in LC patients, thereby improving nitrogen balance and ultimately resulting in better clinical outcomes.[15,18,19] It has also been speculated that the mechanisms involved in the beneficial effects of BCAA might be mediated by their stimulating activity on hepatocyte growth factor, favoring liver regeneration.[20] Previously, we indicated that a BCAA supplement taken p.o. as a late evening snack (LES) prevents the suppression of liver function by transcatheter arterial chemoembolization (TACE) in patients with LC complicated with hepatocellular carcinoma (HCC) during the 2-week period after TACE intake.[21] However, BCAA is a useful drug for the treatment of patients with hepatic malnutrition and encephalopathy,[4] but the influence of BCAA on sleep disturbances has not yet been reported. Therefore, we examined the influence of BCAA supplementation on sleep disturbance symptoms in cirrhotic patients.

METHODS

Patients

WE INVESTIGATED A total of 21 patients at Nagasaki University Hospital, including nine male subjects and 11 female patients from January to June 2009 (Table 1). All patients had LC without HCC, a history of hepatic encephalopathy, chronic renal failure, the use of BCAA supplements, an albumin preparation refill or alcohol drinking. The diagnosis of HCC was based on the findings on contrast enhancement computed tomography scans and magnetic resonance imaging. Hepatic encephalopathy was diagnosed by the clinical findings. All patients had been diagnosed with LC by the laboratory data and imaging findings at the time of entry into the study, and patients were not prescribed BCAA supplementations prior to this study. After balancing both groups for sex, age, Child–Pugh score (CPS), cirrhotic symptom-related score (CSS) and albumin level, patients were randomized into two groups: the BCAA-enriched supplementation group and the control group. No patients in either group suffered overt hepatic encephalopathy or HCC during the observation period. We followed all patients in our hospital.

Laboratory measurements

Laboratory data, anthropometric measurements and survey by questionnaire were performed at the time of entry, 4 weeks later and 8 weeks later. The body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m). Laboratory measurements were as follows: white blood cells (WBC), red blood cells (RBC), platelets (Plt), prothrombin time (PT), blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), albumin (Alb), total cholesterol (TC), cholinesterase (ChE), triglyceride (TG), fasting blood glucose (FPG), ammonia (NH3) and BCAA/tyrosine ratio (BTR).

Survey by questionnaire

We constructed questionnaire items for the evaluation of cirrhotic symptoms. The items, as major symptoms of cirrhotic patients, included hand tremor, appetite loss, muscle cramp of foot, fatigue, decreased strength, anxiety, abdominal fullness, abdominal pain and a feeling of low energy. For each item, we calculated the "impact factor", or the product of proportion of patients who identified the item as a problem (frequency), and the mean importance attributed to that item. The impact factor for each item ranged 0–6. We investigated the total of the impact factors (CSS). We then used the Epworth Sleepiness Scale (ESS)[22] for the evaluation of daytime hypersomnolence. The ESS score ranged 0–24, and a score of 10 or more was indicative of a significant daytime hypersomnolence patient. All patients were assessed by the CSS and ESS at the time of entry, and at 4 and 8 weeks.

Protocol for intake of BCAA-enriched snack

We used a BCAA-enriched snack (Aminoleban EN; Otsuka Pharmaceutical, Tokyo, Japan) for supplemen-

Table 1 Clinical characteristics at the time of patient entry

Character	BCAA group ($n = 12$)	Control group ($n = 9$)
Disease	Viral/alcoholic/other: 8/2/2	Viral/alcoholic/other: 6/2/2
Sex (female : male)	7:5	4:5
Child–Pugh score	6.36 ± 1.65	6.33 ± 1.62
BMI	25.6 ± 5.61	23.8 ± 3.86
Age	66.2 ± 8.21	67.4 ± 9.86
TP	7.29 ± 0.65	6.63 ± 0.804
Alb	3.57 ± 0.83	3.44 ± 0.663
WBC	2972 ± 970	2851 ± 940
RBC	381 ± 82.0	359 ± 71.9
Plt	7.01 ± 2.02	9.28 ± 3.86
PT	65.1 ± 15.7	75.3 ± 21.7
BUN	15.4 ± 5.57	17.3 ± 5.22
Cr	0.745 ± 0.112	0.869 ± 0.186
AST	45.1 ± 22.7	47.0 ± 19.3
ALT	35.9 ± 27.0	38.8 ± 20.9
ALP	431.4 ± 217.7	344.8 ± 150.6
γ-GTP	74.0 ± 99.1	69.3 ± 68.2
TB	1.57 ± 0.735	1.01 ± 0.704
BTR	3.35 ± 1.78	3.50 ± 1.53
NH3	64.9 ± 30.6	75.3 ± 52.3
ChE	176.2 ± 115	150.2 ± 66.4
TC	159.4 ± 30.7	137.1 ± 35.4
TG	63.0 ± 33.2	74.3 ± 23.4
FPG	107.4.8 ± 27.2	115.4 ± 27.2
CSS	17.4 ± 8.1	16.7 ± 10.1
ESS	7.73 ± 4.47	4.67 ± 3.35

Data are shown as the mean ± standard deviation and numbers by the statistical analyses using a Mann–Whitney *U*-test to compare means and the χ^2-test to compare values. Statistically significant differences between the BCAA and control groups were not detected.

Normal values in laboratory tests: ALT (IU/L), 5–40; AST (IU/L), 10–40; γ-GTP (IU/L), <70 in men, <30 in women; TP (g/dL), 6.7–8.3; ALB (g/dL), 4.0–5.0; WBC (/μL), 3500–9000; RBC ($\times 10^4$/μL), 450–580 in men, 380–480 in women; Plt ($\times 10^4$/μL), 14–33; PT (%), 70–130; BUN (mg/dL), 8.0–22.0; Cr (mg/dL), 0.61–1.04 in men, 0.47–0.79 in women; ALP (IU/L), 115–359; LDH (IU/L), 119–229; TB (mg/dL), 0.3–1.5; BTR, 5–9.5; NH3 (μg/dL), <75; ChE (IU/L), 214–466; TC (mg/dL), 128–220; and TG (mg/dL), 38–150; FPG (mg/dL), 70–110.

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCAA, branched chain amino acid; BTR, BCAA/tyrosine ratio; BUN, blood urea nitrogen; ChE, cholinesterase; Cr, creatinine; CSS, cirrhotic symptom-related score; ESS, Epworth Sleepiness Scale; FPG, fasting blood glucose; NH3, ammonia; Plt, platelets; PT, prothrombin time; RBC, red blood cells; TB, total bilirubin; TC, total cholesterol; TG, triglyceride; TP, total protein; WBC, white blood cells; γ-GTP, γ-glutamyl transpeptidase. BMI, bodyweight (kg) / height (m) / height (m).

tation of the LC patients. The treated patients were begun on a daily p.o. BCAA snack (50 g) at 22.00 hours each day from the time of entry and continued after entry for 8 weeks. Aminoleban EN was contained in the snack, which had 13.5 g protein, high levels of BCAA and low levels of the other amino acids, and had a Fischer ratio of 38, and 210 kcal of energy per 50 g pack. The energy supplementation of the BCAA snack was taken as an LES.[21] Patients in the BCAA group carried on intake of BCAA snack after the conclusion of the present study. Furthermore, patients in the control group did not take the BCAA snack. The patients were instructed to maintain a diet containing 30–35 kcal and 1.2–1.3 g of protein/kg of ideal bodyweight/day. In the BCAA group, the patients were educated to adjust their total energy intake by subtracting 210 kcal of the BCAA snack from the meals. In the control group, the control rice ball as an LES provided 210 kcal of energy and 9 g of protein. Nutritional intake was evaluated for all patients by dietitians at the initial period, and at 4 and 8 weeks after initiation of BCAA supplementation.

Statistical analysis

Data were processed on a personal computer and analyzed using StatView ver. 5.0 software program. Differences in the laboratory data were analyzed by the Mann–Whitney *U*-test and χ^2-test for numbers. In addition, the relationship with items was evaluated by the coefficient of correlation. $P < 0.05$ were considered to be statistically significant.

RESULTS

THIS STUDY INCLUDED 21 patients, randomized either to the BCAA group ($n = 12$) or the control group ($n = 9$). The baseline clinical characteristics are outlined in Table 1. The Child–Pugh classifications A, B and C were 6, 5 and 1, and 5, 3 and 1 in the BCAA and control groups, respectively. Hepatitis B viral infection and hepatitis C viral infection in the BCAA and control group were present at equal frequencies. The other etiology describes one of autoimmune hepatitis and one of cryptogenic in the BCAA group and one of primary biliary cirrhosis and one of non-alcoholic steatohepatitis in the control group; all other etiology patients were female. All patients were outpatients during the observation period and were not admitted into the hospital. In the BCAA group, adverse effects of BCAA snack were not identified. The nutritional intake was not different during the initial period or at 4 or 8 weeks after beginning the snack. The LES of the BCAA snack were well

Table 2 Variation of laboratory data from entry to 8 weeks

Laboratory data	8 weeks after entry BCAA	8 week-entry Control	BCAA	Control
BMI	26.1 ± 5.24	23.7 ± 4.00	0.234 ± 0.543	−0.097 ± 0.827
TP	7.18 ± 0.656	6.66 ± 0.835	−0.133 ± 0.42	0.038 ± 0.66
Alb	3.52 ± 0.67	3.43 ± 0.732	−0.017 ± 0.46	−0.013 ± 0.20
WBC	3391 ± 91182	2900 ± 1009	308.3 ± 588	48 ± 560
RBC	385 ± 77.6	353 ± 70.9	6.01 ± 36.6	−6.44 ± 24.4
Plt	7.39 ± 2.44	10.26 ± 5.07	−0.133 ± 0.856	0.978 ± 1.646
PT	66.1 ± 14.7	71.7 ± 15.5	−4.43 ± 27.4	−3.59 ± 8.18
BUN	14.8 ± 4.37	18.3 ± 5.99	−0.58 ± 4.00	1.00 ± 5.41
Cr	0.77 ± 0.112	0.89 ± 0.209	0.23 ± 0.053	0.23 ± 0.063
AST	44.5 ± 15.73	45.0 ± 16.2	−2.00 ± 14.9	−1.50 ± 23.8
ALT	33.3 ± 14.09	29.6 ± 11.0	−2.58 ± 25.0	−9.22 ± 19.3
ALP	412.4 ± 180.1	358.9 ± 150.1	−21.4 ± 141	14.0 ± 64.2
γ-GTP	76.8 ± 103.1	53.1 ± 38.4	6.58 ± 21.3	−16.2 ± 37.2
TB	1.33 ± 0.799	0.80 ± 0.654	−0.25 ± 0.487	−0.21 ± 0.262
BTR	3.69 ± 1.90	3.86 ± 1.50	0.445 ± 1.17	0.364 ± 0.795
NH3	72.6 ± 31.64	63.9 ± 34.0	8.63 ± 21.2	−12.3 ± 18.1
ChE	175.0 ± 94.3	147.7 ± 74.6	2.42 ± 42.4	−2.56 ± 18.7
TC	147.5 ± 30.0	127.6 ± 28.7	−9.67 ± 19.5	−9.56 ± 18.3
TG	52.0 ± 18.2	58.7 ± 42.7	−12.5 ± 12.8	−7.00 ± 23.3
FPG	105.7 ± 73.2	111.6 ± 24.2	21.7 ± 71.3	−45.6 ± 77.6

"8 week-entry" is an increment value in which the data of pre-treatment is pulled from the data of 4 weeks after the observation beginning. Data are shown as the mean ± standard deviation and numbers, with the statistical analyses using a Mann–Whitney *U*-test to compare means. Statistically significant differences between the BCAA and control groups were not detected. Normal values in the laboratory tests were same as in Table 1.

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCAA, branched chain amino acid; BTR, BCAA/tyrosine ratio; BUN, blood urea nitrogen; ChE, cholinesterase; Cr, creatinine; CSS, cirrhotic symptom-related score; ESS, Epworth Sleepiness Scale; FPG, fasting blood glucose; NH3, ammonia; Plt, platelets; PT, prothrombin time; RBC, red blood cells; TB, total bilirubin; TC, total cholesterol; TG, triglyceride; TP, total protein; WBC, white blood cells; γ-GTP, γ-glutamyl transpeptidase. BMI, bodyweight (kg) / height (m) / height (m).

tolerated, and there was a good level of compliance in all patients of the BCAA group.

The laboratory data at 8 weeks and variation between at entry and 8 weeks are shown in Table 2. In the 8-week analyses, the laboratory data were equivalent between the BCAA and control groups. In the variation between the time of entry and 8 weeks in the groups, all laboratory data were equivalent between the BCAA and control groups. The BMI and BMI variation at 8 weeks were not significantly different between the two groups.

However, the laboratory data and BMI did not change between the time of entry and at 8 weeks, and the CSS and ESS were influenced by the BCAA snack (Fig. 1). The CSS (mean ± standard deviation [SD]) was 17.12 ± 7.76 and 16.7 ± 10.1 at the time of entry, 16.08 ± 5.94 and 18.3 ± 10.4 at 4 weeks and 11.7 ± 5.23 and 17.9 ± 10.5 at 8 weeks (Fig. 1a). At 8 weeks in the BCAA group, the score showed a declining trend compared to the time at entry. The differences at the time of entry, at 4 weeks and at 8 weeks appeard to have a declining trend in the BCAA group (Fig. 1b). "4W-entry", "8W-entry" and "8W-4W" were also −1.08 ± 7.60 and 1.667 ± 2.96, −4.42 ± 5.57 and −0.444 ± 2.79, −5.50 ± 10.3 and 1.22 ± 4.32 in the BCAA and control groups, respectively. On the contrary, ESS is recognized to have statistical siginificance following BCAA treatment (Fig. 1c,d). ESS (mean± SD) was 7.67 ± 4.21 and 4.67 ± 3.35 at the time of entry, 5.50 ± 3.15 and 5.78 ± 3.528 at 4 weeks and 4.81 ± 2.19 and 5.78 ± 3.27 at 8 weeks (Fig. 1c). However,the value of ESS was not significantly different at the indicated period in either group. The differences at the time of entry and at 4 and 8 weeks were calculated (Fig. 1b). "4W-entry", "8W-entry" and "8W-4W" were −2.16 ± 3.46 and 1.11 ± 1.364, −0.917 ± 1.78 and −0.00 ± 0.866, −3.08 ± 3.66 and 1.11 ± 1.17 in the BCAA and control groups, respectively. It was understood that a significant decrease of ESS had already begun by the fourth week after the beginning of treat-

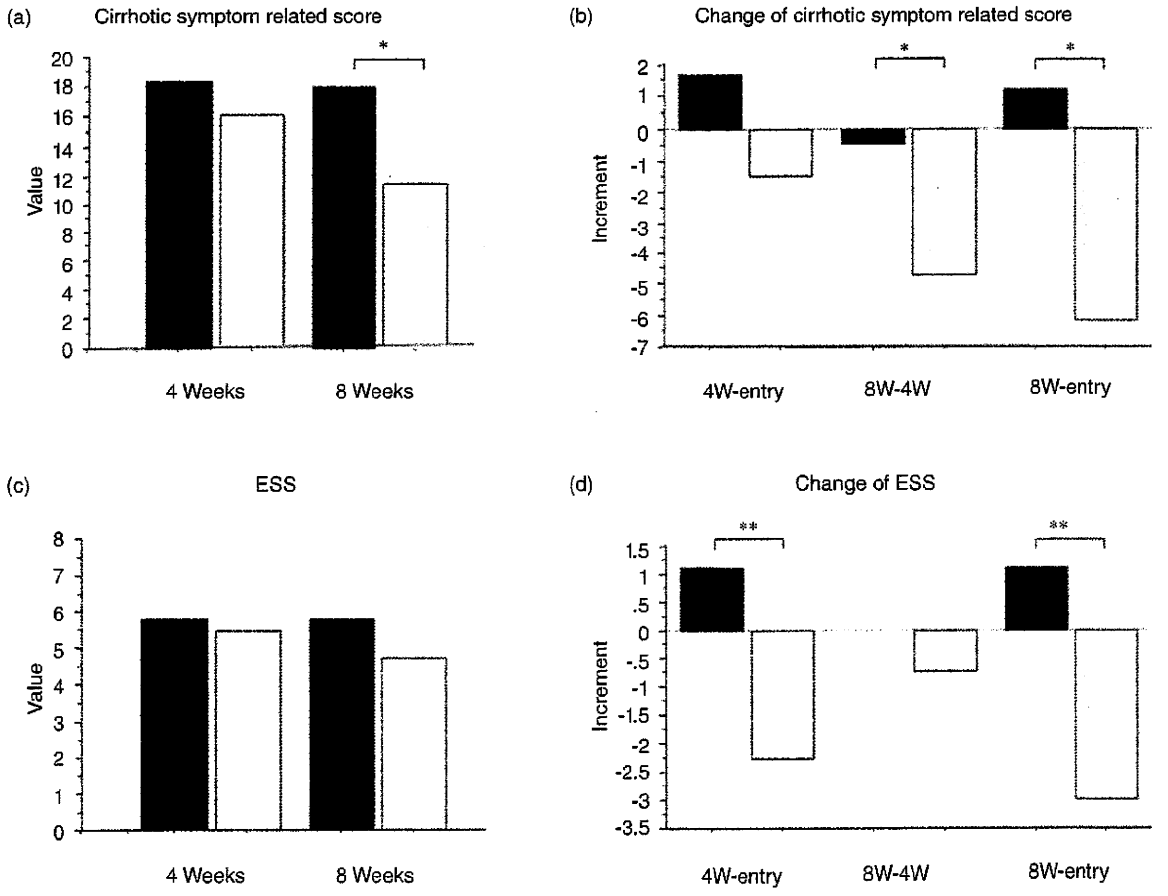

Figure 1 The transition of cirrhotic symptom-related score and Epworth Sleepiness Scale (ESS) during the follow-up period. The values of cirrhotic symptom-related score (a) and ESS (c) at 4 weeks and 8 weeks. Changes in the cirrhotic symptom-related score (b) and ESS (d). "4W-entry" is an increment value in which the data of pre-treatment was pulled from the data of 4 weeks after the observation began. "8W-entry" and "8W-4W" were also calculated as the differences between the two indicated periods. The black box indicates the mean value in the observation group. The gray box indicates the mean value in the branched-chain amino acid group. $^{*}P < 0.1$. $^{**}P < 0.05$. $^{***}P < 0.01$.

ment. The decrease became more significant by the eighth week after beginning the treatment (Fig. 1d).

Finally, we examined the factors that were related to ESS at the time of entry. At entry, the CSS was positively related with the CPS ($R = 0.545$), but was not related to ESS (Fig. 2a,b). Additionally, ESS was not related to the CPS (Fig. 2c), or to items of the CSS and laboratory data (BMI, WBC, RBC, Plt, PT, BUN, Cr, AST, ALT, γ-GTP, ALP, TB, TP, Alb, TC, ChE, TG, FPG, NH3 and BTR) at the time of entry. In the comparison at the time of entry and 8 weeks after, ESS was improved only in the BCAA treatment group, and we experienced only one subject who had ESS or CSS that became more negative in the control group (Fig. 2d).

There were four cases that had an ESS score of 10 or more in the BCAA group. The ESS at the time of entry and at 4 and 8 weeks was 17, 7 and 5 in a 50-year-old female patient who suffered from hepatitis B virus infection (CPS = 6 and CSS = 15), 10, 6 and 6 in a 55-year-old male patient who suffered from excessive alcohol consumption (CPS = 7 and CSS = 30), 12, 13 and 9 in a 71-year-old female patient who suffered from hepatitis B virus infection (CPS = 5 and CSS = 23) and 10, 5 and 5 in a 73-year-old male who suffered from excessive alcohol consumption (CPS = 5 and CSS = 6). Three of four patients had a diminished ESS score at 4 weeks compared with that at the time of entry, all patients were improved to an ESS of under 10. The ESS at entry, 4 and

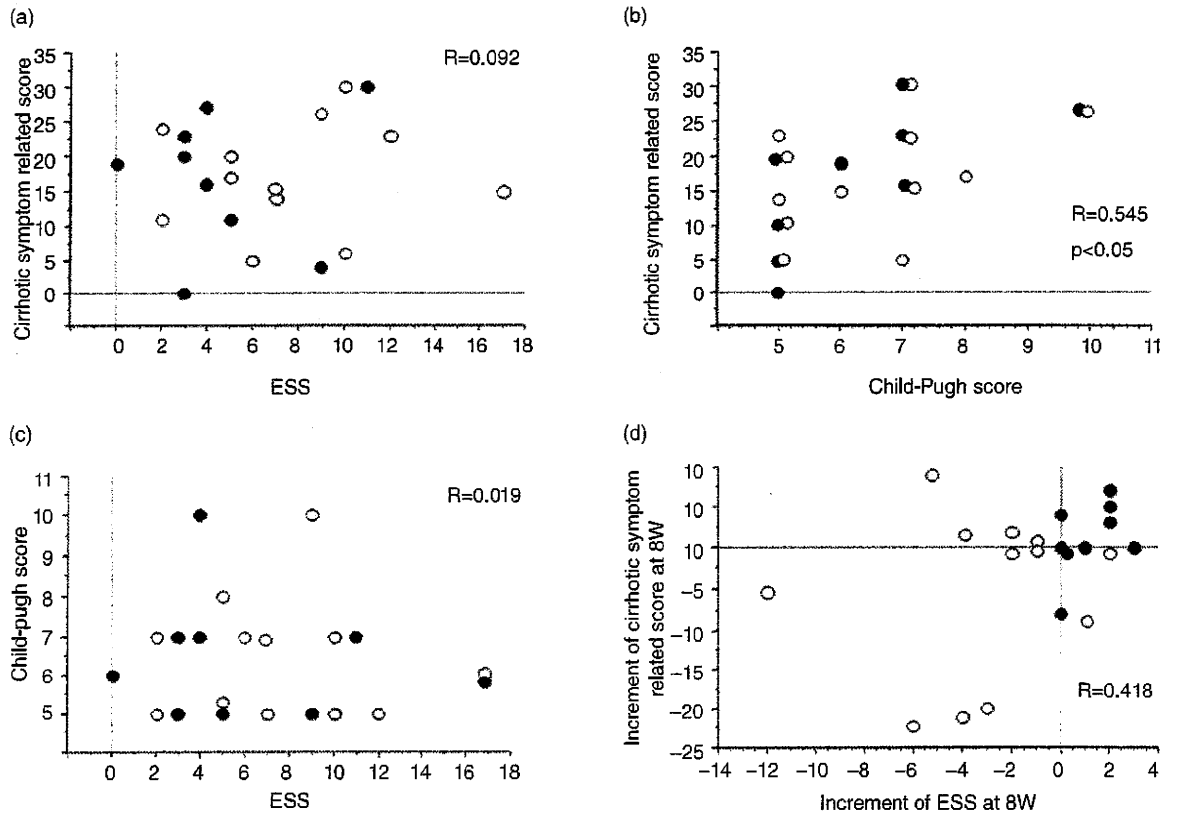

Figure 2 The scatter graph of Epworth Sleepiness Scale (ESS), cirrhotic symptom-related and Child–Pugh scores. The scatter graph indicates the cirrhotic symptom-related score and ESS (a), cirrhotic symptom-related score and the Child–Pugh score (b), the Child–Pugh score and the ESS score (c) and increments of the cirrhotic symptom-related score from 8 weeks after entry and the ESS from 8 weeks after entry (d). The increment value was the difference from 8 weeks after entry. Black circles indicate the patients in the observation group. Gray circles indicate the patients in the BCAA group. The relationship between the cirrhotic symptom-related score and the Child–Pugh score was positively and statistically correlated.

8 weeks was 11, 13 and 12 in a 77-year-old male patient who suffered from hepatitis C virus infection (CPS = 7 and CSS = 30) in the control group.

DISCUSSION

IN THE PRESENT study, we found that a BCAA snack, which was taken p.o. as an LES, improved the ESS for cirrhotic patients without encephalopathy. This beneficial result was recognized in the short term, at 4 weeks after beginning of treatment, and demonstrated the availability of BCAA supplementation for cirrhotic patients with sleep disturbances. However, the CSS was positively correlated with the CPS at the time of patient entry, but we were not able to identify the item that was related to ESS.

The frequency of sleep disturbance in cirrhotic patients was not evaluated in this study, because we did not assign a normal healthy control group. An ESS of 10 or higher, indicating significant daytime hypersomnolence, was present in five of 21 cirrhotic patients (23.8%), and all cirrhotic patients had a mean ESS of 6.38. In a previous report,[23] it was described that a mean ESS of 5.6 in 144 healthy control cases was lower than the Parkinson's disease group. In another study,[8] it was reported that cirrhotic patients had a mean ESS of 6.66 (6.17 in healthy control subjects), and 15.7% of the subjects had an ESS of greater than 10 (12.9% of healthy controls). In a primary biliary cirrhosis study,[5] it was reported that patients had a mean ESS of 9 (5 in healthy controls), and more than 50% of these subjects had an ESS of greater than 10 (15% of healthy controls). We

believe that our ESS data from cirrhotic patient is consistent with previous studies. In addition, all of the patients with significant daytime hypersomnolence demonstrated an improvement in their symptoms after regularly consuming the BCAA snack in this study.

The mechanism of sleep disturbance in cirrhotic patients is still unclear. It has been considered that sleep disturbance is an early sign of hepatic encephalopathy and a symptom of minimal hepatic encephalopathy (MHE), which is characterized by cognition dysfunction without overt encephalopathy.[24] There is no current consensus on how MHE should be diagnosed. However, there are several requirements for the diagnosis of MHE: a normal mental status at the time of clinical examination; documentation of neurological impairment by multiple methods; and the exclusion of other disturbances that may cause neurological impairment.[25] In this study, our patients had a normal mental status and did not have other neurological impairments, which were not documented. Therefore, our patients were not diagnosed with MHE. However, it is likely that a thorough evaluation for a relationship with MHE and sleep disturbance is necessary. Previous reports suggested that the psychometric test was not correlated with ESS,[26] but there was no relationship between sleep and cognitive performance either at baseline or in relation to treatment.[27] There was a positive correlation with the MHE and CPS,[25] but there was no correlation between sleep disturbance and CPS.[1] In this study, ESS was not related to liver function or the CSS. It has been considered that MHE is a cause for sleep disturbance in cirrhotic patients, but another inducer of sleep disturbance may be the cirrhosis itself.

It has been speculated in several studies that the relationship between sleep disturbance and MHE may be due to the deterioration of the circadian rhythm.[1,24] In particular, melatonin and its metabolite regulate circadian rhythm and are involved in hepatic metabolism and cause sleep disturbances for the delayed sleep phase, in which the melatonin levels peak significantly later in cirrhotic patients.[28] However, it was reported that cirrhosis does not shift the circadian phase of plasma fibrinolysis.[29] Because we did not evaluate sleep phases in the present cirrhotic patients, it is necessary to evaluate abnormal circadian variations in future. However, the BCAA snack was effective for ameliorating sleep disturbances in cirrhotic patients. Tryptophan, an aromatic amino acid, elevated in cirrhotic patients, is the precursor for the neurotransmitter 5-hydroxytryptamine (5-HT), which is involved in fatigue and sleep.[30,31] In a previous report,[31] it was suggested that BCAA supplementation may help to counteract the effects of an increase in free plasma tryptophan. It has been suggested that the plasma BCAA concentrations may influence brain function and affect appetite, physical and mental fatigue, mental performance and physical endurance.[32] In other reports, it has been described that the p.o. intake of BCAA may reduce tryptophan uptake and 5-HT synthesis and release, thereby delaying fatigue.[30] Recently, it was reported that fatigue in patients with liver disease is significantly associated with excessive daytime sleepiness and a high average ESS.[6] In this study, ESS was not related with our indicated data, but it will be necessary to examine the participations of the factor, for instance, fatigue, in future studies.

In the present study, no difference in BTR was found between the BCAA and control groups. We speculated that the cause is the short treatment period. In previous reports, the elevation of BTR was observed at 3 months[33] and 1 year[34] after BCAA supplementation (Aminoleban EN). However, the elevation of BTR in the BCAA group at 8 weeks might be caused by an increased number of entry cases.

Because benzodiazepines should not be used for cirrhotic patients with sleep disturbances,[35] the availability of the BCAA snack for cirrhotic patients with sleep disturbances must be verified. Previous reports showed that BCAA can be a psychotropic drug which directly acts on the central nervous system.[4] Restless legs syndrome and obstructive sleep apnea syndrome are known to be the cause of sleep disturbances in cirrhotic patients.[6,36] The cause of sleep disturbance and the relationship with prognosis and sleep disturbance will therefore be examined in future studies.

REFERENCES

1 Cordoba J, Cabrera J, Lataif L *et al.* High prevalence of sleep disturbance in cirrhosis. *Hepatology* 1998; 27: 339–45.
2 Carskadon MA, Brown ED, Dement WC. Sleep fragmentation in the elderly: relationship to daytime sleep tendency. *Neurobiol Aging* 1982; 3: 321–7.
3 Bonnet MH. Effect of sleep disruption on sleep, performance, and mood. *Sleep* 1985; 8: 11–19.
4 Watanabe A. Cerebral changes in hepatic encephalopathy. *J Gastroenterol Hepatol* 1998; 13: 752–60.
5 Newton JL, Gibson GJ, Tomlinson M *et al.* Fatigue in primary biliary cirrhosis is associated with excessive daytime somnolence. *Hepatology* 2006; 44: 91–8.
6 Newton JL, Jones DE, Henderson E *et al.* Fatigue in non-alcoholic fatty liver disease (NAFLD) is significant and associates with inactivity and excessive daytime sleepiness

but not with liver disease severity or insulin resistance. *Gut* 2008; **57**: 807–13.
7 Kim K, Uchiyama M, Okawa M *et al.* An epidemiological study of insomnia among the Japanese general population. *Sleep* 2000; **23**: 41–7.
8 Mostacci B, Ferlisi M, Baldi Antognini A *et al.* Sleep disturbance and daytime sleepiness in patients with cirrhosis: a case control study. *Neurol Sci* 2008; **29**: 237–40.
9 Lautz HU, Selberg O, Korber J *et al.* Protein-calorie malnutrition in liver cirrhosis. *Clin Investig* 1992; **70**: 478–86.
10 Muller MJ, Lautz HU, Plogmann B *et al.* Energy expenditure and substrate oxidation in patients with cirrhosis: the impact of cause, clinical staging and nutritional state. *Hepatology* 1992; **15**: 782–94.
11 Crawford DH, Shepherd RW, Halliday JW *et al.* Body composition in nonalcoholic cirrhosis: the effect of disease etiology and severity on nutritional compartments. *Gastroenterology* 1994; **106**: 1611–17.
12 Nutritional status in cirrhosis. Italian Multicentre Cooperative Project on Nutrition in Liver Cirrhosis. *J Hepatol* 1994; **21**: 317–25.
13 Nielsen K, Kondrup J, Martinsen L *et al.* Nutritional assessment and adequacy of dietary intake in hospitalized patients with alcoholic liver cirrhosis. *Br J Nutr* 1993; **69**: 665–79.
14 Kajiwara K, Okuno M, Kobayashi T *et al.* Oral supplementation with branched-chain amino acids improves survival rate of rats with carbon tetrachloride-induced liver cirrhosis. *Dig Dis Sci* 1998; **43**: 1572–9.
15 Yoshida T, Muto Y, Moriwaki H *et al.* Effect of long-term oral supplementation with branched-chain amino acid granules on the prognosis of liver cirrhosis. *Gastroenterol Jpn* 1989; **24**: 692–8.
16 Marchesini G, Dioguardi FS, Bianchi GP *et al.* Long-term oral branched-chain amino acid treatment in chronic hepatic encephalopathy. A randomized double-blind casein-controlled trial. The Italian Multicenter Study Group. *J Hepatol* 1990; **11**: 92–101.
17 Marchesini G, Bianchi G, Merli M *et al.* Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. *Gastroenterology* 2003; **124**: 1792–801.
18 Nakaya Y, Okita K, Suzuki K *et al.* BCAA-enriched snack improves nutritional state of cirrhosis. *Nutrition* 2007; **23**: 113–20.
19 Moriwaki H, Miwa Y, Tajika M *et al.* Branched-chain amino acids as a protein- and energy-source in liver cirrhosis. *Biochem Biophys Res Commun* 2004; **313**: 405–9.
20 Tomiya T, Omata M, Fujiwara K. Significance of branched chain amino acids as possible stimulators of hepatocyte growth factor. *Biochem Biophys Res Commun* 2004; **313**: 411–16.
21 Takeshita S, Ichikawa T, Nakao K *et al.* A snack enriched with oral branched-chain amino acids prevents a fall in albumin in patients with liver cirrhosis undergoing chemoembolization for hepatocellular carcinoma. *Nutr Res* 2009; **29**: 89–93.
22 Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 1991; **14**: 540–5.
23 Suzuki K, Miyamoto T, Miyamoto M *et al.* Excessive daytime sleepiness and sleep episodes in Japanese patients with Parkinson's disease. *J Neurol Sci* 2008; **271**: 47–52.
24 Bajaj JS. Minimal hepatic encephalopathy matters in daily life. *World J Gastroenterol* 2008; **14**: 3609–15.
25 Ortiz M, Jacas C, Cordoba J. Minimal hepatic encephalopathy: diagnosis, clinical significance and recommendations. *J Hepatol* 2005; **42** (Suppl): S45–53.
26 Vignatelli L, Mattarozzi K, Zanatta C *et al.* Cognitive function and Epworth Sleepiness Scale in "minimal" hepatic encephalopathy. *Eur J Neurol* 2001; **8**: 369.
27 Spahr L, Coeytaux A, Giostra E *et al.* Histamine H1 blocker hydroxyzine improves sleep in patients with cirrhosis and minimal hepatic encephalopathy: a randomized controlled pilot trial. *Am J Gastroenterol* 2007; **102**: 744–53.
28 Steindl PE, Finn B, Bendok B *et al.* Disruption of the diurnal rhythm of plasma melatonin in cirrhosis. *Ann Intern Med* 1995; **123**: 274–7.
29 Piscaglia F, Hermida RC, Siringo S *et al.* Cirrhosis does not shift the circadian phase of plasma fibrinolysis. *Am J Gastroenterol* 2002; **97**: 1512–17.
30 Newsholme EA, Blomstrand E. Branched-chain amino acids and central fatigue. *J Nutr* 2006; **136**: 274S–6S.
31 Castell LM, Yamamoto T, Phoenix J *et al.* The role of tryptophan in fatigue in different conditions of stress. *Adv Exp Med Biol* 1999; **467**: 697–704.
32 Hawkins RA, O'Kane RL, Simpson IA *et al.* Structure of the blood-brain barrier and its role in the transport of amino acids. *J Nutr* 2006; **136**: 218S–26S.
33 Aoyama K, Tsuchiya M, Mori K *et al.* Effect of a late evening snack on outpatients with liver cirrhosis. *Hepatol Res* 2007; **37**: 608–14.
34 Habu D, Nishiguchi S, Nakatani S *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**: 312–18.
35 Blei AT, Cordoba J. Hepatic encephalopathy. *Am J Gastroenterol* 2001; **96**: 1968–76.
36 Franco RA, Ashwathnarayan R, Deshpandee A *et al.* The high prevalence of restless legs syndrome symptoms in liver disease in an academic-based hepatology practice. *J Clin Sleep Med* 2008; **4**: 45–9.

ELSEVIER

Macrophage-Dominant Sialadenitis in Human T-Cell Leukemia Virus Type I–Associated Myelopathy After Living-Donor Liver Transplantation

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ABSTRACT

A 64-year-old man who suffered from human T-cell leukemia virus type I (HTLV-I)–associated myelopathy (HAM) after living-donor liver transplantation (LDLT) for liver cirrhosis due to hepatitis C virus infection complained of xerostomia. Although exocrine function test results were positive, autoantibodies including anti-SS-A/SS-B antibodies and sialography showed negative findings. Labial salivary gland biopsy revealing infiltration of 60 counts of mononuclear cells (MNCs) in minor salivary glands led to a diagnosis of Sjögren's syndrome-like sialadenitis. Immunohistochemistry demonstrated dominant CD68 staining and major histocompatibility complex class II on the surface of infiltrating MNCs. Herein we have reported a rare condition of macrophage-dominant sialadenitis in a patient with HAM after LDLT.

BOTH hepatitis C virus (HCV) and human T-cell leukemia virus type I (HTLV-I) have been reported to be associated with the onset of Sjögren's syndrome (SS).[1,2] Because HCV infection demonstrates exocrine dysfunction along with sialadenitis, the American-European Consensus Group for SS excluded HCV infection in the diagnosis of SS.[3] We have previously reported that an epidemiological study showed a high prevalence of SS in anti–HTLV-I antibody–positive subjects.[4] In this case, a complication of HTLV-I–associated myelopathy (HAM) after living-donor liver transplantation (LDLT) has recently been reported.[5] Herein, we have additionally reported the emergence of unusual sialadenitis in this patient.

CASE REPORT

The complication of HAM in this patient was already reported by Soyama et al.[5] Briefly, LDLT was performed for a patient who had decompensated liver cirrhosis due to HCV infection in August 2002. Both the patient and his younger sister donor were seropositive for anti–HTLV-I antibody. Immediately after LDLT in October 2002, interferon (IFN) α-2b and ribavirin were administered after we confirmed recurrence of the HCV infection, but HAM appeared 18 months after LDLT. Although pegylated IFN α-2b and ribavirin were administered for 48 weeks against the HCV infection, no response was observed to the recurrent active hepatitis.

When the patient was admitted in September 2008, xerostomia was newly detected. The new clinical manifestations of HAM included spastic gait and bladder symptoms. Elevation of aspartate aminotransferase (53 IU/L), alanine aminotransferase (47 IU/L), and immunoglobulin (Ig)G (2630 mg/dL) were observed with normal total bilirubin (0.7 mg/dL). Type IV collagen and quantitative HCV ribonucleoprotein were elevated at 290 ng/mL (normal, ≤140) and 7.1 log IU/mL (normal, undetected) with reduced total branched chain amino acids (285 μmol/L; normal, 379–688). A liver biopsy, which had resulted in a hospital admission in September 2008, showed chronic hepatitis with fibrous enlargement of the portal area, inflammatory cell infiltration, and piecemeal necrosis. The relative copy number of HTLV-I against

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All authors declare no conflicts of interest in this article.

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β-globin in the peripheral blood sample was $2.56 \times 10^2/10^4$ cells by real-time polymerase chain reaction. Along with xerostomia, both the Saxon test (1.1 g/2 minutes; <2 g, positive) and Schirmer test (3 mm/5 minutes; <5 mm, positive) were positive with negative results for anti-SS-A/SS-B antibodies and sialography. However, minor salivary gland biopsy (Fig 1A) demonstrated more than 60 counts of mononuclear cell (MNC) infiltration, which were confirmed as dominantly macrophages. Although the patient showed signs of xerostomia, positive exocrine dysfunction, and MNC infiltration into the minor salivary gland (MSG), SS was excluded according to the criteria determined by the American-European Consensus Group.[3] Immunohistochemistry using monoclonal antibodies for MSG demonstrated positive staining of CD68 on the infiltrating MNCs (Fig 1B). Compared with the prevalence of CD68, the prevalence of CD4 (Fig 1C) or CD8 (Fig 1D) was less than that of CD68, macrophage. Major histocompatibility (MHC) class II was found in human tonsil as a positive control (Fig 1E) and MNCs in the MSG of this patient (Fig 1F). Written informed consent for the use of the biopsy specimen was obtained from the patient.

DISCUSSION

HCV-related SS has been reported to be characterized by a high prevalence of cryoglobulinemia with a low frequency of anti-SS-A/SS-B antibodies.[6] In our case, sialadenitis without SS-related autoantibodies was compatible with the characteristics of HCV-related SS. However, HCV infection usually shows infiltration of CD4+ T lymphocytes into the MSG, which is incompatible with the present macrophagic infiltration. Furthermore, it has previously been reported that IFNs have the potential to cause autoimmune diseases, such as autoimmune thyroid diseases, systemic lupus erythematosus, rheumatoid arthritis, or SS.[7,8] Unoki et al reported that administration of IFN-alpha-2b for a patient with type C chronic active hepatitis induced SS with sicca symptoms and elevation of autoantibodies, suggesting that IFN per se has a potential to form autoimmune disorders in patients with viral hepatitis.[9]

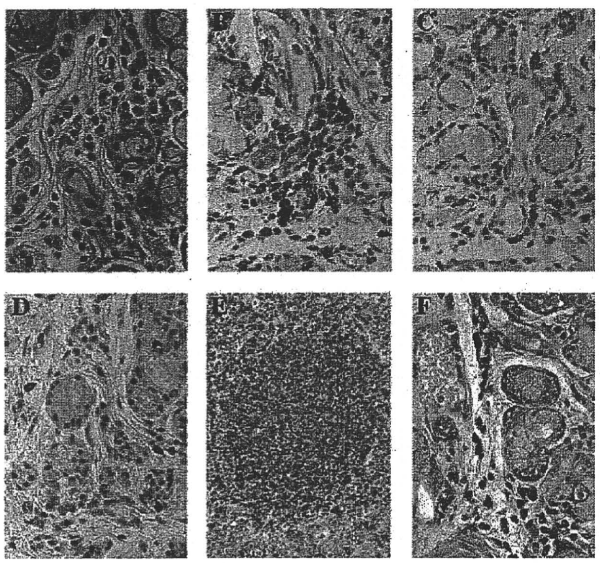

Fig 1. Phenotypic markers expressed in the minor salivary gland (MSG). Immunohistochemistry was performed for formalin-fixed, paraffin-embedded sections (3-μm thick) from the MSG using the streptavidin-biotin method. Primary antibodies were used as follows: **(A)** hematoxylin-eosin staining, **(B)** CD68, **(C)** CD4, **(D)** CD8, **(E)** MHC class II staining in human tonsil (positive control), and **(F)** MHC class II staining of the MSG of the patient. (Original magnification for A–D and F, ×200; E ×100.) Hematoxylin was used as a counterstain.

With regard to HTLV-I infection, prognosis of HTLV-I–positive renal transplant recipients has been previously reported,[10] observing that both living-related and cadaveric kidneys from HTLV-I carriers may be used for HTLV-I–seropositive recipients because of the low occurrence of adult T-cell leukemia. HTLV-I is also one of the candidates to trigger sialadenistis. We have previously reported a high prevalence of SS among patients with HAM.[2] However, the predominant phenotype of MNCs in HAM-SS patients was CD4+ T lymphocytes, which was similar to the type of MNCs in HTLV-I–seronegative SS patients.

Previously Ishiguro et al[11] established a rat model of HTLV-I infection in which massive foamy macrophages infiltrated the spinal cord and clinical manifestations of the rat resembled those of HAM patients. Meanwhile, our patient showed macrophage-dominant MNC infiltration into the MSG. Although the pathogenesis of the rat model might be different from that of human HAM, because lymphocytic infiltration is an apparent characteristic of HAM patients, an unrecognized trigger might have induced infiltration of macrophages into the MSG in our patient.

Graft-versus-host disease (GVHD) is considered to be a candidate cause of sialadenitis. Fujiwara et al[12] have previously reported sialadenitis in experimental GVHD in an animal model, in which nonirradiated mice were injected with spleen cells developing chronic GVHD. In their report, sialadenitis was observed predominantly with CD4+ T lymphocytes, although with a low frequency of macrophages, B cells, or plasma cells. However, chronic GVHD has rarely been reported after LDLT. Sun et al[13] reported a case of GVHD at 4 months after cadaveric liver transplantation. In their report, the patient showed gastrointestinal symptoms, which were determined to be T-lymphocyte infiltration based on a colonic biopsy.

In summary, the mechanism by which sialadenitis is induced remains to be clarified. However, both active hepatitis and HAM have the potential for viral-induced recruitment of mononuclear infiltration. Furthermore, double viral infection may provoke a strong elimination reaction compared with a single viral infection. Although an antiviral reaction is considered to be increased by innate immunity through Toll-like receptors,[14] intense antigen-presentation capacity might be yielded by induction of macrophages.

REFERENCES

1. Ramos-Casals M, Loustaud-Ratti V, De Vita S, et al: Sjögren syndrome associated with hepatitis C virus: a multicenter analysis of 137 cases. Medicine (Baltimore) 84:81, 2005

2. Nakamura H, Kawakami A, Tominaga M, et al: Relationship between Sjögren's syndrome and human T-lymphotropic virus type I infection: follow-up study of 83 patients. J Lab Clin Med 135:139, 2000

3. Vitali C, Bombardieri S, Jonsson R, et al: Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 61:554, 2002

4. Terada K, Katamine S, Eguchi K, et al: Prevalence of serum and salivary antibodies to HTLV-1 in Sjögren's syndrome. Lancet 344:1116, 1994

5. Soyama A, Eguchi S, Takatsuki M, et al: Human T-cell leukemia virus type I-associated myelopathy following living-donor liver transplantation. Liver Transpl 14:647, 2008

6. Ramos-Casals M, De Vita S, Tzioufas AG: Hepatitis C virus, Sjögren's syndrome and B-cell lymphoma: linking infection, autoimmunity and cancer. Autoimmun Rev 4:8, 2005

7. Wilson LE, Widman D, Dikman SH, et al: Autoimmune disease complicating antiviral therapy for hepatitis C virus infection. Semin Arthritis Rheum 32:163, 2002

8. Bhakri H, Sriskandan K, Davis T, et al: Recombinant gamma interferon and autoimmune thyroid disease. Lancet 2:457, 1985

9. Unoki H, Moriyama A, Tabaru A, et al: Development of Sjögren's syndrome during treatment with recombinant human interferon-alpha-2b for chronic hepatitis C. J Gastroenterol 31:723, 1996

10. Nakamura N, Tamaru S, Ohshima K, et al: Prognosis of HTLV-I-positive renal transplant recipients. Transplant Proc 37: 1779, 2005

11. Ishiguro N, Abe M, Seto K, et al: A rat model of human T lymphocyte virus type I (HTLV-I) infection. 1. Humoral antibody response, provirus integration, and HTLV-I-associated myelopathy/tropical spastic paraparesis-like myelopathy in seronegative HTLV-I carrier rats. J Exp Med 176:981, 1992

12. Fujiwara K, Sakaguchi N, Watanabe T: Sialoadenitis in experimental graft-versus-host disease. An animal model of Sjögren's syndrome. Lab Invest 65:710, 1991

13. Sun B, Zhao C, Xia Y, et al: Late onset of severe graft-versus-host disease following liver transplantation. Transpl Immunol 16:250, 2006

14. Kawai T, Akira S: Antiviral signaling through pattern recognition receptors. J Biochem 141:137, 2007

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Perioperative synbiotic treatment to prevent infectious complications in patients after elective living donor liver transplantation. A prospective randomized study

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KEYWORDS:
Synbiotic therapy;
Living donor liver transplantation;
Infectious complication

Abstract

BACKGROUND: Although the effect of synbiotic therapy using prebiotics and probiotics has been reported in hepatobiliary surgery, there are no reports of the effect on elective living-donor liver transplantation (LDLT).

METHODS: Fifty adult patients undergoing LDLT between September 2005 and June 2009 were randomized into a group receiving 2 days of preoperative and 2 weeks of postoperative synbiotic therapy (*Bifidobacterium breve*, *Lactobacillus casei*, and galactooligosaccharides [the BLO group]) and a group without synbiotic therapy (the control group). Postoperative infectious complications were recorded as well as fecal microflora before and after LDLT in each group.

RESULTS: Only 1 systemic infection occurred in the BLO group (4%), whereas the control group showed 6 infectious complications (24%), with 3 cases of sepsis and 3 urinary tract infections with *Enterococcus* spp (P = .033 vs BLO group). No other type of complication showed any difference between the groups.

CONCLUSIONS: Infectious complications after elective LDLT significantly decreased with the perioperative administration of synbiotic therapy.

The bowel has bacterial flora, in which 100 billion bacteria are present with a weight of 1 kg.[1] Bacterial translocation can occur if the intact environment is compromised, leading to the provocation of several cytokine networks and multiple organ failure in the end.[2–5] Liver transplant recipients in particular usually have a long history of liver disease and suffer portal hypertension, which leads to malnutrition.[6] Therefore, the mucosa of their bowels could be atrophic and more susceptible to bacterial translocation, which leads to endotoxemia and multiple organ failure.[7–9]

"Synbiotic therapy" is the medical term for comprehensive prebiotic therapy combined with probiotic therapy.[10] It has been used for the amelioration of stool character, the suppression of toxic substances, and immunomodulation for various infectious diseases and is reported to provide good therapeutic efficacy.[9–11] Probiotics are bacteria that can provide beneficial effects by maintaining the balance of resident bacteria in the bowel, such as bifidobacteria and lactobacteria.[12,13] Generally, probiotics increase the intestinal motility and stabilize the intestinal barrier for bacterial location.[14,15] Furthermore, probiotics, which are living bac-

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Table 1 Patient characteristics

Variable	BLO group (n = 25)	Control group (n = 25)	*P*
Age (y)	56 (33–66)	57 (25–68)	NS
Men/women	13/12	16/9	NS
Primary disease	LC-C (n = 9)	LC-C (n = 13)	NS
	LC-B (n = 5)	LC-B (n = 7)	
	LC-Al (n = 3)	Caroli disease (n = 1)	
	LC-AIH (n = 2)	FHF (n = 1)	
	PSC (n = 3)	LC-Al (n = 1)	
	PBC (n = 2)	PV thrombus (n = 1)	
	LC unknown (n = 1)	PSC (n = 1)	
ABO incompatibility	9 (36%)	4 (16%)	NS
GV/SLV ratio (%)	39 (24.8–61)	41.5 (23.6–57)	NS
MELD score	15 (2–34)	16 (4–41)	NS
Concomitant HCC	12	12	NS

Data are expressed as median (range) or as number (percentage).

BLO = *Bifidobacterium breve, Lactobacillus casei*, and galactooligosaccharides; FHF = fulminant hepatic failure; GV = graft volume; HCC = hepatocellular carcinoma; LC-AIH = liver cirrhosis due to autoimmune hepatitis; LC-Al = liver cirrhosis due to alcohol intoxication; LC-B = liver cirrhosis due to hepatitis B virus; LC-C = liver cirrhosis due to hepatitis C virus; LC unknown = liver cirrhosis of unknown origin; MELD = Model for End-Stage Liver Disease; PBC = primary biliary cirrhosis; PSC = primary sclerosing cholangitis; PV = portal vein; SLV = standard liver volume.

teria, can protect the innate immune system with cytokine modulation. By contrast, prebiotics are an ingredient made from food and delivered to the large bowel, which can stimulate the proliferation of beneficial bacteria such as bifidobacteria. Prebiotics can reach the colon without any transformation and serve as nutrition for probiotics.[2] Synbiotic therapy reduces the rate of infection after pylorus-preserving pancreaticoduodenectomy,[16] major hepatectomy for bile duct cancer,[17] deceased-donor whole-liver transplantation,[18,19] and acute pancreatitis.[20] However, no reports have indicated whether infectious complication can be reduced by synbiotic therapy after living-donor liver transplantation (LDLT). Because LDLT is always partial transplantation, postoperative portal hypertension is higher in LDLT compared with whole-liver transplantation.[21]

Therefore, this prospective randomized controlled study was conducted to determine if synbiotic therapy during the perioperative period is effective in reducing infectious complications for recipients undergoing LDLT.

Methods

Patients

This prospective study was approved by the local institutional review board at Nagasaki University Hospital, and written informed consent was obtained from all patients.

Fifty liver transplant recipients at Nagasaki University Hospital treated between June 2005 and June 2009 were enrolled in this study. The α error was set at 5%, with power of 80%. According to previous reports, infectious complications occur in 40% of liver transplant recipients and could be reduced by synbiotic therapy to 10%.[18,19] Therefore, the calculated sample size was 25 patients for each group.

Patients were randomly assigned to groups receiving (n = 25) or not receiving (n = 25) synbiotic therapy. The characteristics of the patients are shown in **Table 1**. The primary endpoint of this study was to the reduction of infectious complications after LDLT with synbiotic therapy.

Liver transplantation

All partial liver grafts were preserved in University of Wisconsin solution and implanted using a piggyback technique, as previously described.[22] Surgeons experienced in microscopic surgery anastomosed all the hepatic arteries with the aid of a surgical microscope. Graft selection was based on the results of a volumetric study using computed tomography to obtain a ratio of graft volume to standard liver volume of >35% in the recipients. All patients received intravenous prophylaxis with amoxicillin and cefotiam for 4 days as a standard protocol. Empiric therapy was initiated in the event of infection, and subsequently antibiotics were narrowed on the basis of the resistance index.

A dual or triple immunosuppressive regimen was used, which included tacrolimus or cyclosporine A, prednisolone, and/or mycophenolate mofetil. Patients with compromised renal function were administered induction therapy with interleukin-2 antibodies. Only biopsy-proven rejections were treated if clinical and laboratory signs mandated steroid bolus treatment. Rituximab (anti-CD20 antibody) was used preoperatively for immunosuppression in ABO-incompatible patients.

Age, gender, primary liver disease, ABO incompatibility, median graft volume versus standard liver volume, Model for End-Stage Liver Disease score at time of LDLT,

and concomitant hepatocellular carcinoma were compared between the group receiving synbiotic therapy and the control group.

Subsequently, at 24 hours after LDLT, all patients received enteral nutrition with Elental (Ajinomoto Pharmaceutical Ltd, Tokyo, Japan), which is an elemental diet, through a tube jejunostomy made during liver transplantation. The initial infusion rate at 1 kcal/mL was 20 mL/h, and if tolerated the rate was increased 60 mL/h until sufficient oral intake was possible. The composition of Elental has been described elsewhere.[23]

Synbiotic therapy

All patients had started the oral administration of Yakult BL antiflatulent (Yakult Honsha, Tokyo, Japan), containing 20 mg of living *Lactobacillus casei* strain Shirota, 15 mg of living *Bifidobacterium breve* strain Yakult, and galactooligosaccharides 15 g/d (Oligomate 55; Yakult Honsha) 3 times per day from 2 days before elective LDLT, continued for 2 weeks after LDLT via either a tube jejunostomy or orally. Usually, both prebiotics and probiotics were taken with 10 mL of tap water. We selected this formula of synbiotics on the basis of a previous report on major hepatectomy.[16]

The rates of infectious complications and patient survival were recorded, and stool cultures were also performed.

Statistical analysis

All data are expressed as median values with ranges. Statistical analysis was performed using the Mann-Whitney U test for continuous values and the χ^2 test for categorical values. A statistically significant difference was defined as a P value $< .05$. StatView version 5.0 (Abacus Concepts, Berkeley, CA) was used for all statistical analyses.

Results

All patients tolerated synbiotic therapy throughout the study period. There was no difference in the patient characteristics between the groups (Table 1). Figure 1 shows the result of cultured bacteria in the feces. Generally, *Escherichia* spp, *Enterobacter* spp, and *Klebsiella* spp were regarded as normal bacterial flora in the stool. There was no significant pattern of the change of bacterial species between the groups. However after LDLT under immunosuppression, *Enterococcus* spp became evident in both groups in about 25% of the patients.

Table 2 that infectious complication occurred after LDLT in 6 of 25 of the patients in the control group (24%) and in 1 of 25 (4%) in group receiving synbiotic therapy ($P < .05$). In particular, the rate of urinary infection was higher without synbiotic therapy. The rate of intra-abdominal infection was not statistically different. *Enterococcus* spp and methicillin-resistant *Staphylococcus aureus* were the main bacteria related to the infection. The postoperative date of infection varied. Some infectious complication occurred after the termination of synbiotic therapy.

Table 3 shows that there was no significant difference between the groups in other complications after LDLT. In addition, there were no differences in the intensive care unit period, hospitalized period, and mortality rate between the groups.

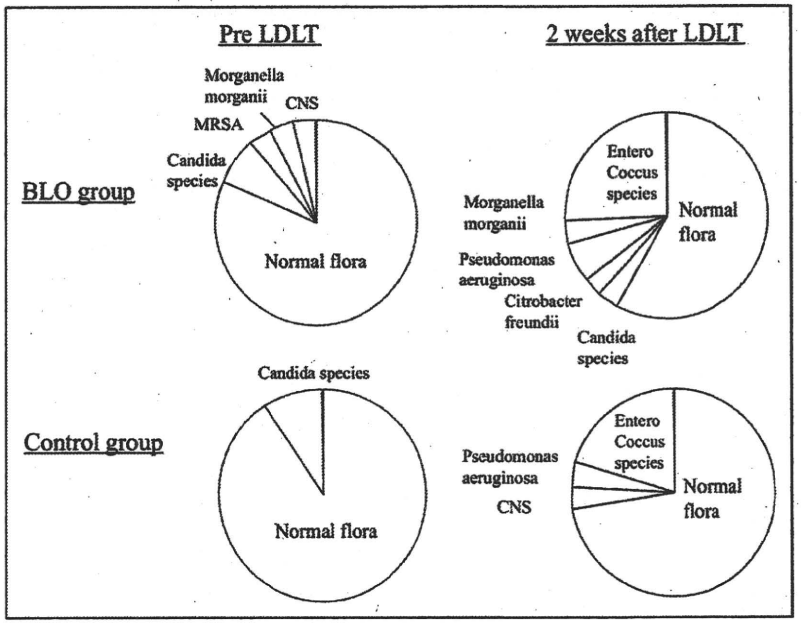

Figure 1 Bacterial profile in fecal culture. Cultured bacteria in the feces of the patients undergoing LDLT in each group. BLO = *Bifidobacterium breve*, *Lactobacillus casei*, and galactooligosaccharides; CNS = coagulase-negative staphylococci; MRSA = methicillin resistant *Staphylococcus aureus*.

Table 2 Infectious complications after LDLT

Variable	BLO group (n = 25)	Control group (n = 25)	*P*
Type of infection	1 catheter infection (POD 19)	3 sepsis (PODs 11, 10, and 9) 3 urinary tract infections (PODs 7, 8, and 5)	<.05
Bacteria cultured in blood	1 *Enterobacter asburiae* (POD 19)	2 MRSA (PODs 10 and 9) 1 MRSA + *Candida glabrata* (POD 11) 3 *Enterococcus faecium* (PODs 7, 8, and 5)	
Intra-abdominal infection	1 (4%) *Klebsiella oxytoca* + *Enterococcus faecium* (POD 19)	2 (8%) 1 *Enterobactor asburiae* (POD 19) 1 *Enterococcus faecium* (POD 14)	NS

BLO = *Bifidobacterium breve, Lactobacillus casei,* and galactooligosaccharides; MRSA = methicillin resistant *Staphylococcus aureus*; POD = postoperative day.

Comments

This prospective randomized study demonstrated that synbiotic therapy successfully reduced the rate of infectious complications after LDLT, which has a greater chance to induce temporary portal hypertension leading to bacterial translocation. The portal venous pressure after LDLT should have been elevated in the current series of patients, because the graft volume versus standard liver volume ratio was about 40%.[21] Therefore, synbiotic therapy may be potentially more effective in patients after LDLT than deceased-donor liver transplantation. In addition, LDLT is partial transplantation, in which liver regeneration should occur to support the patient's life. Infection itself was reported to reduce the magnitude of liver regeneration, so synbiotic therapy should be used for the patients undergoing LDLT.[24]

The patients in the present study received enteral nutrition, which has been shown to reduce the rate of infection from 29% to 14%.[25,26] This is probably why the rate of infection in this study was lower than in previous reports with synbiotic therapy. In addition, the rate of acute cellular rejection was not changed by synbiotic therapy. In a previous study, the rate of acute cellular rejection was reduced from 44% to 7% by enteral nutrition after whole-liver transplantation.[27] There was no difference in the rejection rate, even though there were more ABO-incompatible LDLT patients in the synbiotic group than in the control group.

Methicillin-resistant *S aureus* and *Enterococcus* spp were the principle bacteria causing sepsis, although gram-negative gut-derived bacteria are thought to be found in septic patients. Although there was no explanation for the gram-positive bacteria in this series, *Enterococcus* spp were frequently observed as the dominant bacteria after LDLT in the feces.[28] Immunosuppression and the duration of our antibiotic use might have cause *Enterococcus* sepsis in partial liver transplant recipients. In addition, the reduction of urinary tract infections was reported in a previous study, consistent with the current data, indicating that synbiotic therapy is likely to be responsible for the reduction of urinary tract infection.[29] Previous authors have speculated that in addition to their impact on bacterial translocation, probiotics act via several other mechanisms. For instance, they can reduce and eliminate potentially pathogenic microorganisms, reduce and eliminate various toxins and mutagens from the urine and feces, modulate innate and adaptive immune defense mechanisms, promote apoptosis, and release numerous nutrients, antioxidants, and growth factors from consumed fibers. These functions might all be important for the reduction of infections in surgical patients. However, a definite mechanism regarding the reduction of urinary tract infection awaits further investigation.[3,25,29,30]

In conclusion, infectious complications after LDLT were significantly decreased with synbiotic therapy. It is possible to achieve ecologic liver transplantation using synbiotic therapy while maintaining an intact environment in the body.

Table 3 Other complications

Variable	BLO (n = 25)	Control (n = 25)	*P*
Others	2 ACR 3 CMV 1 HAT 1 HPS 1 TMA 1 adrenal insufficiency	3 ACR 3 CMV 1 HAT 1 HPS 1 NOMI	NS
ICU period (days)	7 (4–35)	7 (2–48)	NS
Hospitalized period (days)	40 (16–132)	33 (16–97)	NS
Mortality	3	3	NS

Data are expressed as median (range) or as numbers.

ACR = acute cellular rejection; BLO = *Bifidobacterium breve, Lactobacillus casei,* and galactooligosaccharides; CMV = cytomegalovirus; HAT = hepatic arterial thrombus; HPS = hemophagocytic syndrome; ICU = intensive care unit; NOMI = nonocclusive mesenteric ischemia; TMA = thrombolytic microangiopathy.

References

1. Backhed F, Ley RE, Sonnenburg JL, et al. Host-bacterial matualism in the human intestine. Science 2005;25:1915–20.