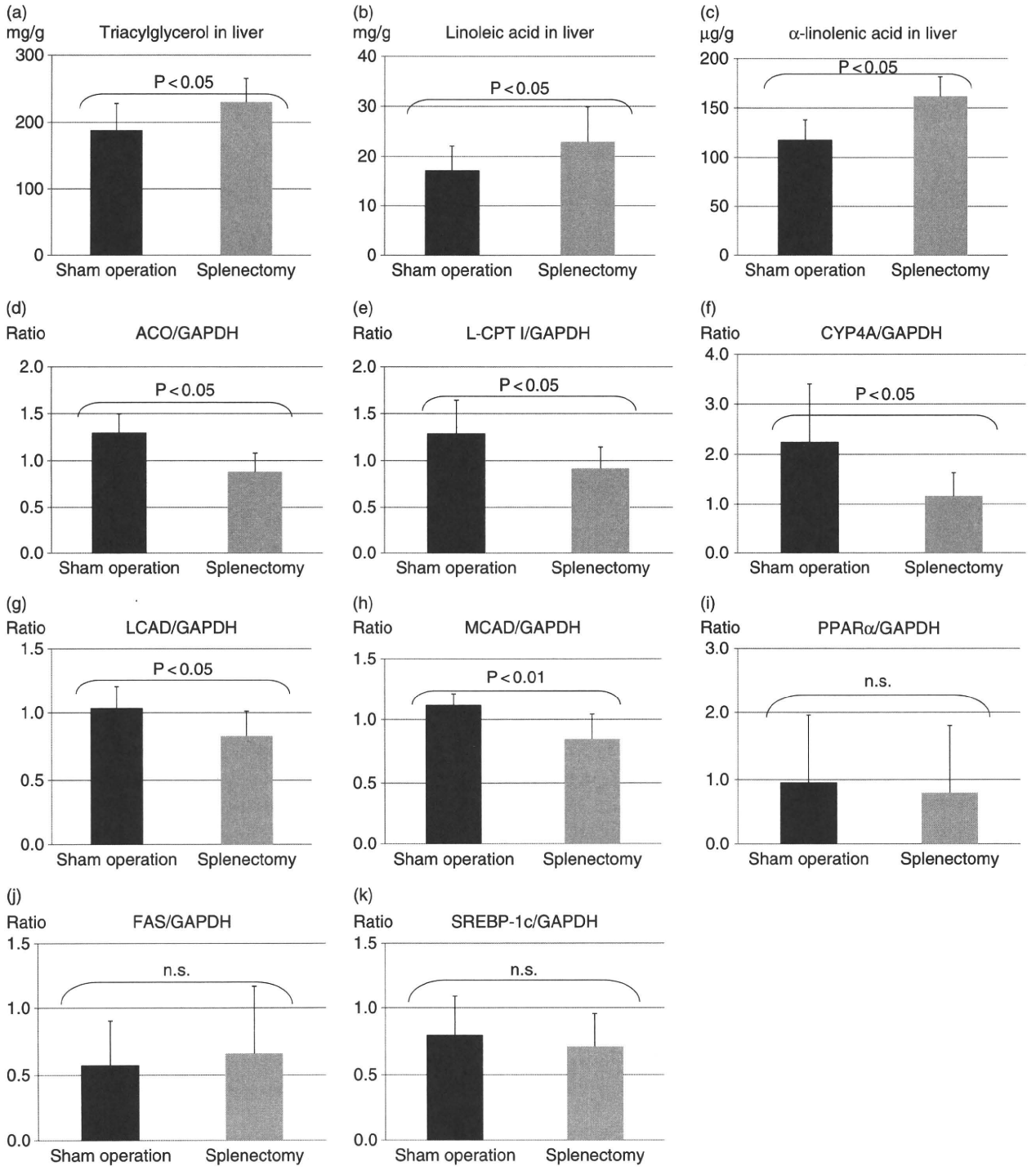

Figure 4 Liver TG (a) and linoleic acid (b) and α-linolenic acid in liver (c). mRNA expression of ACO (d), L-CPTI (e), CYP4A (f), LCAD (g), MCAD (h), PPAR-α (i), FAS (j) and SREBP-1c (k) in liver between sham operation and splenectomy group. Data are means ± standard deviation. ACO, acyl-coenzyme A oxidase; CYP4A, cytochrome P450 4A; FAS, fatty acid synthase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LCAD, long-chain acyl-coenzyme A dehydrogenase; L-CPTI, liver carnitine palmitoyl-coenzyme A transferase I; MCAD, medium-chain acyl-coenzyme A dehydrogenase; n.s., no significant difference; PPAR-α, peroxisome proliferator-activated receptor-α; SREBP-1c, sterol regulatory element-binding protein-1c; TG, triglyceride.

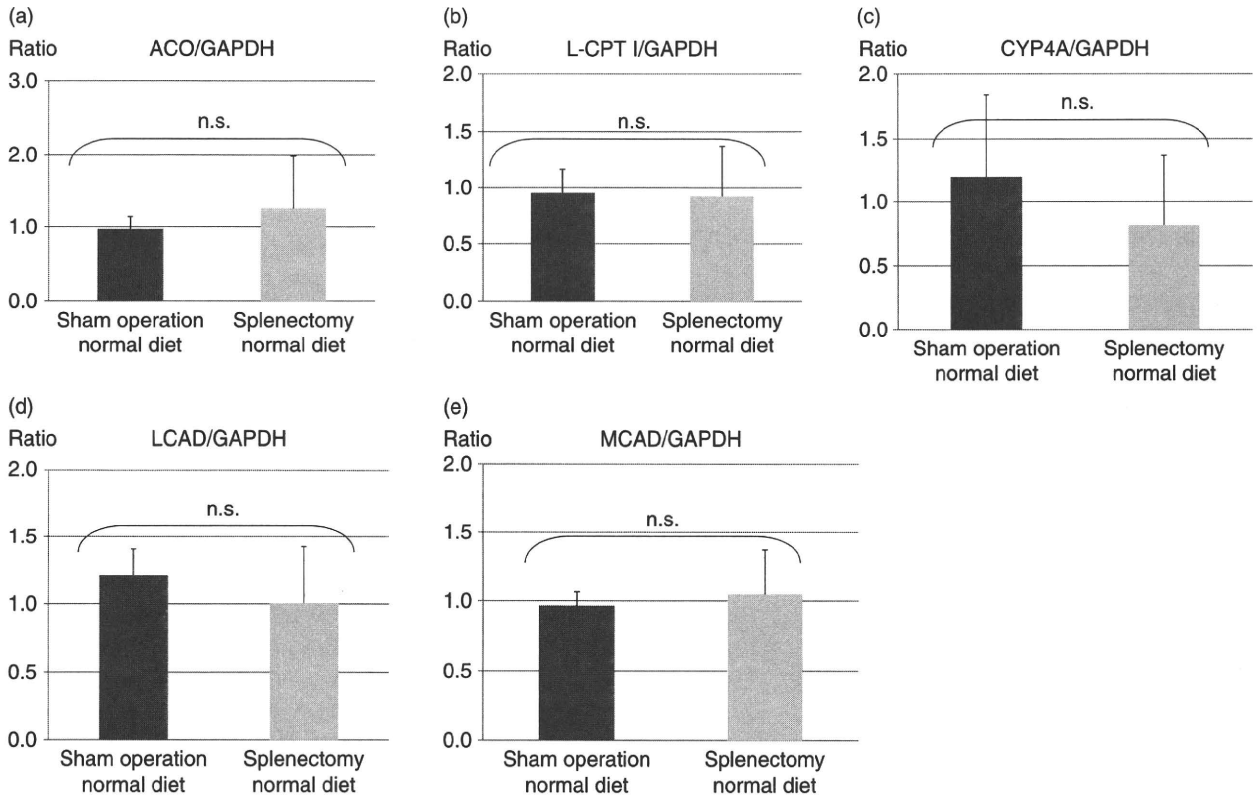

Figure 5 mRNA expression of ACO (a), L-CPTI (b), CYP4A (c), LCAD (d) and MCAD (e) in liver between sham operation and splenectomy normal diet group. Data are means ± standard deviation. n.s., no significant difference. ACO, acyl-coenzyme A oxidase; CYP4A, cytochrome P450 4A; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LCAD, long-chain acyl-coenzyme A dehydrogenase; L-CPTI, liver carnitine palmitoyl-coenzyme A transferase I; MCAD, medium-chain acyl-coenzyme A dehydrogenase; n.s., no significant difference.

From our results, exacerbation of fatty liver changes is thus likely caused by decreases in fatty acid metabolism. In other words, decreased liver fatty acid metabolism in the splenectomy group would increase liver essential fatty acids, and thus increase liver TG.

As our model is an undernutrition steatosis model,[12,13] no significant differences were seen between groups in terms of blood lipids. However, according to previous reports using normal and high-fat diets, splenectomy may worsen blood lipid metabolism.[9–11] This may also have developed due to an exacerbation of fatty liver changes by splenectomy.

It remains unclear the amount of essential fatty acids in the liver and the working. Our result showed that the quantity of essential fatty acids was proportional to TG and was inverse proportional to fibrosis area in the end-stage of NASH. These results suggest that the quantity of essential fatty acid in the liver decreases when NASH progresses to cirrhosis.

As detailed studies on NASH etiology and pathogenesis have not yet been conducted, no clear statement can be made about whether splenectomy can improve NASH pathogenesis. However, our study focusing on and comparing liver fibrosis and preneoplastic lesions found that both were clearly reduced by splenectomy. In cases of liver fibrosis due to NASH, splenectomy may improve liver fibrosis. In addition, in humans, the incidence of HCC in NASH over a 19.5-year observation period has been reported as 0–2.8%.[22–25] Splenectomy has potential as a therapy to reduce this incidence.

The past human study[26] showed that older age and advanced fibrosis were important risk factors for HCC, and that the steatosis in NASH patients without HCC was severer than that with HCC. These results suggest that the quantity of TG in the liver decreases when NASH progresses to cirrhosis. In other words, it is suggested that liver fibrosis decreases but steatosis is severer

when some factors improve NASH in the end stage. It is consistent with our result.

Based on the above, aggressive treatment with splenectomy may be worthwhile in NASH patients in whom symptoms and morbidity are primarily associated with liver fibrosis and cirrhosis as major determinants of prognosis. On the other hand, in NASH patients in whom symptoms and morbidity are due to obesity, poorly controlled lifestyle disease or atherosclerotic disease, which are determinants of the prognosis, splenectomy may exacerbate the lifestyle-related disease, so particular caution must be taken.

In conclusion, these findings suggest that spleen plays an important regulatory role in the fibrosis, preneoplastic lesion and lipid metabolism of liver in a rat CDAA model.

ACKNOWLEDGMENTS

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

A novel transcatheter arterial infusion chemotherapy using iodized oil and degradable starch microspheres for hepatocellular carcinoma: a prospective randomized trial

Takahiro Yamasaki · Satoe Hamabe · Issei Saeki · Yohei Harima · Yuhki Yamaguchi · Koichi Uchida · Shuji Terai · Isao Sakaida

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Abstract
Background We designed a novel transcatheter arterial infusion chemotherapy (TAI) using iodized oil (lipiodol) and degradable starch microspheres (DSM) for hepatocellular carcinoma (HCC) patients. In this study, we investigated the efficacy of TAI using lipiodol and DSM in a prospective randomized trial.
Methods We randomly divided 45 patients with HCC into 3 groups: TAI using lipiodol (lipiodol group, $n = 15$), TAI using DSM (DSM group, $n = 15$), and TAI using lipiodol and DSM (lipiodol + DSM group, $n = 15$). In the lipiodol group, a mixture of cisplatin and lipiodol was administered. In the DSM group, a mixture of cisplatin and DSM was administered. In the lipiodol + DSM group, a mixture of cisplatin and lipiodol was administered, followed by DSM.
Results The response rates were 40% in the lipiodol group, 53.4% in the DSM group, and 80% in the lipiodol + DSM group, respectively. The response rate tended to improve in the lipiodol + DSM group (lipiodol group vs. lipiodol + DSM group, $P = 0.07$). The median progression-free survival time was 177 days in the lipiodol group, 287 days in the DSM group, and 377 days in the lipiodol + DSM group. The progression-free survival in the lipiodol + DSM group was significantly better than those in the DSM group ($P = 0.020$) and the lipiodol group ($P = 0.035$). There were no serious adverse effects among the 3 groups.
Conclusions TAI using lipiodol and DSM was superior to TAI using lipiodol only and TAI using DSM only because of improvements in therapeutic effects and progression-free survival.

Keywords Hepatocellular carcinoma · Transcatheter arterial infusion chemotherapy · Iodized oil · Degradable starch microspheres · Randomized trial

T. Yamasaki (✉) · S. Hamabe · I. Saeki · Y. Harima · Y. Yamaguchi · K. Uchida · S. Terai · I. Sakaida
Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minamikogushi, Ube, Yamaguchi 755-8505, Japan
e-mail: t.yama@yamaguchi-u.ac.jp

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer in the world [1]. Deaths due to HCC are increasing in almost all countries worldwide, including Japan [2–4]. Recent advancements in several therapeutic techniques such as hepatic resection, percutaneous ethanol injection, radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE), sorafenib, and transplantation have improved the prognosis of HCC patients [5–10].

Of these treatments, TACE has become one of the most popular for HCC patients. TACE in Japan has generally used several anticancer agents, iodized oil (lipiodol) and gelatin sponge particles [11]. On the other hand, polyvinyl alcohol (PVA), drug-eluting beads (DEB), and embospheres have been used as embolizing agents in Europe and the United States [12]. Studies prior to 2000 failed to prove a survival benefit of TACE in the treatment of HCC [13, 14]. However, the survival benefit of TACE was proven by meta-analysis in recent reports [15, 16]. In addition, with the development of the microcatheter, the catheter can be inserted in the segmental or subsegmental hepatic artery, and segmental or subsegmental TACE has been reported to be a useful treatment [7, 17]. On the other hand, transcatheter arterial infusion chemotherapy (TAI) using an emulsion of lipiodol and

an anticancer agent (without gelatin sponge particles) has usually been performed for HCC patients in whom the catheter could not be inserted in the targeted segment or a feeding artery was not detected in the tumor. In addition, TAI without gelatin sponge particles has been also used for HCC in high-risk patients (for example, main portal vein occlusion, Child–Pugh B or C) [18]. We have also experienced that repeated TACE therapy is not possible due to obstruction of the hepatic artery in HCC patients. Therefore, we have been performing segmental or subsegmental TACE for selected HCC patients. However, it has been reported that the effect of TAI using lipiodol was lower than that of TACE in local tumor control [19]. Many interventional radiologists desire a novel therapy that is both more effective than TAI using lipiodol in local tumor control and is less damaging to the hepatic artery than TACE.

Degradable starch microspheres (DSM) were developed to provide transient occlusion of small arteries [20, 21]. The duration of occlusion in the hepatic arteries by DSM is limited to 80 min [22]. Several studies of metastatic liver tumors indicate that intra-arterial therapy with DSM and an anticancer agent improves the therapeutic effects compared with therapy using an anticancer agent alone [22–24]. However, few studies have evaluated TAI using DSM in HCC patients [25–27].

Given this background, we designed a novel TAI using lipiodol and DSM for use in HCC patients [28]. After a mixture of an anticancer agent and lipiodol is injected, DSM is administered until stasis or reflux of the arterial flow. We postulate that TAI using two occlusion materials may be beneficial because of the tight interruption of blood supply for HCC. In this study, we investigated the efficacy of a novel TAI using lipiodol and DSM in a prospective randomized trial.

Materials and methods

Patients

The eligibility criteria for inclusion in this study were as follows: (1) age 20–80 years; (2) Child–Pugh score of A or B; leukocyte count $\geq 3000/mm^3$; (3) hemoglobin level ≥ 9.5 g/dL; (4) platelet count $\geq 50000/mm^3$; (5) serum creatinine level <1.2 mg/dL; (6) total bilirubin <3.0 mg/dL; (7) locally nodular disease without extrahepatic metastasis and/or vascular tumor thrombosis (portal vein, hepatic vein, and bile duct); (8) no indication for surgical resection and local ablation, or patients rejected surgical resection; and (9) Eastern Cooperative Oncology Group (EOGG) performance status of 0–1 [29].

We studied 45 patients with HCC who had been admitted to the Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, between February 2006 and May 2008. We randomly divided the patients into 3 groups before the angiography: TAI using lipiodol (lipiodol group, $n = 15$), TAI using DSM (DSM group, $n = 15$), and TAI using lipiodol and DSM (lipiodol + DSM group, $n = 15$). The primary outcome measure was tumor response. Secondary outcome measures included progression-free survival and toxicity (Fig. 1). HCC was diagnosed on the basis of imaging results (hyperattenuation in the arterial phase and hypoattenuation in the portal-venous phase) and elevated serum levels of α-fetoprotein (AFP) and/or des-γ-carboxyprothrombin (DCP).

Patients provided their written informed consent before participating in the study, which was approved by the Institutional Review Board of Yamaguchi University Hospital.

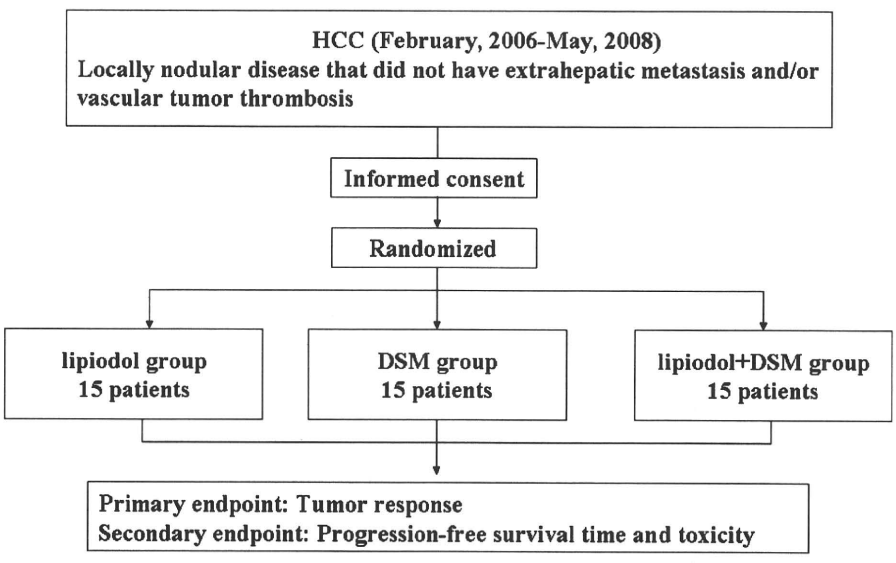

Fig. 1 Study design. We randomly divided patients into 3 groups: transcatheter arterial infusion chemotherapy (TAI) using lipiodol (lipiodol group, $n = 15$), TAI using degradable starch microspheres (DSM) (DSM group, $n = 15$), and TAI using lipiodol and DSM (lipiodol + DSM group, $n = 15$)

Table 1 Clinical profiles of the 45 patients with hepatocellular carcinoma

Clinical characteristics	Lipiodol group ($n = 15$)	DSM group ($n = 15$)	Lipiodol + DSM group ($n = 15$)	P value
Age	69.5 ± 4.4	68.0 ± 7.9	69.3 ± 9.1	NS
Gender (male/female)	12/3	12/3	10/5	NS
HCV Ab(+)/HBs Ag(+)/others	12/2/1	12/2/1	11/3/1	NS
Child–Pugh A/B	11/4	8/7	12/3	NS
Maximum tumor size (mm)	27.1 ± 16.2	33.6 ± 12.8	27.7 ± 15.1	NS
Tumor stage I/II/III[a]	2/4/9	0/2/13	0/7/8	NS
Number of tumors 1/2/3/4/5≦	2/2/3/1/7	1/2/2/2/8	1/3/3/3/5	NS
Previous treatment (yes/no)	15/0	15/0	15/0	NS

DSM degradable microspheres, *NS* not significant

[a] According to the criteria of the Liver Cancer Study Group of Japan

Table 1 summarizes the clinical profiles of the patients in the 3 groups. There were no significant differences between the 3 groups with regard to age, gender ratio, proportion of patients with hepatitis B virus and hepatitis C virus infections, Child–Pugh score, maximum tumor size, tumor stage, number of tumors, or previous treatment. Tumor stage was determined according to the criteria of the Liver Cancer Study Group of Japan [30, 31]. Tumor staging was based on the following 3 parameters (T factor): solitary tumor, <2 cm in diameter and no vessel invasion. Stage I was defined as one fulfilling all of the above 3 criteria (T1); stage II as one fulfilling 2 of the above 3 criteria (T2); stage III as one fulfilling 1 of the above 3 criteria (T3); stage IV A as one fulfilling none of the above 3 criteria (T4) with no distant metastasis or as one with any T factor with lymph node metastasis; and stage IV B as one with any T factor with distant metastasis.

Embolization technique

Hepatic angiography was performed with a 4-French (4-Fr) or 5-Fr angiographic catheter. After digital subtraction angiography (DSA), angiography combined with a computed tomography (angio-CT) [32] system using a Somatom plus 4 (Siemens, Erlagen, Germany) was performed to carefully evaluate HCC tumors. In this study, a fine-powder formulation of cisplatin (IA-call; Nippon Kayaku Co., Tokyo, Japan) was used as the anticancer agent. The dose of cisplatin was limited to 80 mg. According to the tumor vascularization and distribution, TAI was performed by selectively introducing a catheter into the right or left hepatic artery or a segmental branch of the hepatic artery. Gelatin sponge particles were not used in this study.

In the lipiodol group, a mixture of cisplatin and lipiodol (Lipiodol Ultra Fluid; Andre Guerbet, Paris, France) was administered through the tumor-supplying vessels. In the DSM group, a mixture of cisplatin and emulsion obtained by mixing DSM (Spherex; Yakult Honsha Co., Tokyo, Japan) and contrast agent was administered. If this procedure was insufficient, lipiodol or DSM alone was injected until stasis and reflux were achieved.

A mixture of cisplatin and lipiodol was administered in the lipiodol + DSM group. After that point, emulsion obtained by mixing DSM and contrast agent was injected until stasis and reflux were achieved.

The serotonin antagonist ondansetron hydrochloride was administered intravenously as an antiemetic prior to treatment in all 3 groups. To prevent kidney damage, adequate hydration was ensured before and after the treatment by an intravenous drip infusion of 1000–2000 mL of an infusion solution.

After the treatment, a follow-up examination including CT, tumor marker measurement, and serum biochemistry, was performed, first at 1 month after treatment completion and subsequently every 3–4 months. In principle, the same transcatheter arterial treatments were repeated unless the tumors progressed, when a follow-up CT examination showed new lesions in the liver or regrowth of previously treated tumors.

Response and toxicity evaluation

The antitumor effect was assessed by dynamic CT 1 month or more after treatment. The response was classified according to the Liver Cancer Study Group of Japan criteria [30]. In the response evaluation criteria, lipiodol accumulation in the tumors is regarded as an indication of necrosis because significant positive correlations have been reported between lipiodol accumulation observed on CT images and the necrotic regions in the resected tumors examined pathologically after TACE and TAI [33–35]. Therapeutic effect IV (TE IV) is defined as the disappearance or 100% necrosis of all tumors, and TE III as a greater than 50% reduction in tumor size and/or greater than 50% necrosis. TE I is defined as a greater than 25% increase in tumor size. TE II is defined as disease that does not qualify for classification as TE IV, III, or I.

When repeated TAI was performed, the greatest antitumor effect was assessed as the final response.

The severity of adverse reactions was evaluated during the first treatment cycle according to the Common Terminology Criteria for Adverse Events v.4.0 (CTCAE v.4.0) [36].

Statistical analysis

The data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using the unpaired *t* test and the Mann–Whitney *U* test as appropriate. Progression-free survival and cumulative survival were calculated by the Kaplan–Meier method [37] and significance was determined by the log-rank test. Progression-free survival time was defined as the interval between the first TAI after randomization and death or the progression of the last follow-up period. Survival time was defined as the interval between the first TAI after randomization and death or the last follow-up period. The follow-up period ended on April 30, 2010. Statistical significance was defined as a $P < 0.05$.

Results

Information on the anticancer agent and embolizing agents

The median doses of cisplatin at first TAI in the lipiodol group, the DSM group, and the lipiodol + DSM group were 64.3 ± 22.0 mg (20–80 mg), 59.4 ± 20.0 mg (20–80 mg), and 60.5 ± 20.1 mg (10–80 mg), respectively. There was no significant difference in cisplatin dose among the 3 groups. In the lipiodol group, the dose of lipiodol at first TAI was 4.8 ± 2.0 mL (1–8 mL). In the DSM group, the dose of DSM at first TAI was 1164.6 ± 1013.1 mg (120–3000 mg). In the lipiodol + DSM group, the doses of lipiodol and DSM at first TAI were 4.1 ± 2.0 mL (0.5–8 mL) and 426.6 ± 404.8 mg (60–1500 mg), respectively.

Response to therapy

The total number of treatment courses was 23 with a mean of 1.5 courses per patient (range 1–5 courses) in the lipiodol group, 29 with a mean of 1.9 courses per patient (range 1–6 courses) in the DSM group, and 29 with a mean of 1.9 courses per patient (range 1–6 courses) in the lipiodol + DSM group.

Table 2 shows the final response to therapy. In the lipiodol group ($n = 15$), 4 (26.7%), 2 (13.3%), 4 (26.7%), and 5 (33.3%) patients exhibited TE VI, III, II, and I, respectively [response rate (patients with TE VI and III/all patients) = 40%; complete response (CR) rate (patients with TE VI/all patients) = 26.7%]. In the DSM group ($n = 15$), 4 (26.7%), 4 (26.7%), 7 (46.6%), and 0 (0%) patients exhibited TE IV, III, II, and I, respectively (response rate = 53.4%; CR rate = 26.7%). In the lipiodol + DSM group ($n = 15$), 6 (40%), 6 (40%), 2 (13.3%), and 1 (6.7%) patient exhibited TE IV, III, II, and I, respectively (response rate = 80%; CR rate = 40%). The response rate tended to improve in the lipiodol + DSM group (lipiodol group vs. lipiodol + DSM group, $P = 0.07$; Mann–Whitney *U* test). However, no significant differences were seen between the 3 groups (lipiodol group vs. DSM group, $P = 0.21$; DSM group vs. lipiodol + DSM group, $P = 0.25$; Mann–Whitney *U* test).

Table 2 Response to therapy

Group	TE[a]				Response rate[b] (CR rate[c])
	IV	III	II	I	
Lipiodol group ($n = 15$)	4	2	4	5	40% (26.7%)
DSM group ($n = 15$)	4	4	7	0	53.4% (26.7%)
Lipiodol + DSM group ($n = 15$)	6	6	2	1	80% (40%)

TE therapeutic effect, *CR* complete response, *DSM* degradable microspheres

[a] According to the criteria of the Liver Cancer Study Group of Japan

[b] Response rate, patients with TE IV and III/all patients

[c] CR rate, patients with TE IV/all patients

[#] Lipiodol group versus DSM group, $P = 0.21$

[##] DSM group versus lipiodol + DSM group, $P = 0.25$

[###] Lipiodol group versus lipiodol + DSM group, $P = 0.07$

Progression-free survival

Figure 2 shows the progression-free survival rates for the 3 groups. The 1- and 2-year progression-free survival rates in the lipiodol group were 13 and 13%, respectively. The 1-year progression-free survival rate was 27% in the DSM group. The 1-, 2-, and 3-year progression-free survival rates in the lipiodol + DSM group were 53, 13, and 7%, respectively. The median progression-free survival times were 177 days in the lipiodol group, 287 days in the DSM group, and 377 days in the lipiodol + DSM group. No significant difference in progression-free survival was seen between the lipiodol group and the DSM group ($P = 0.515$). On the other hand, progression-free survival in the lipiodol + DSM group was significantly better than that in the DSM group ($P = 0.020$) and the lipiodol group ($P = 0.035$).

Survival

In the lipiodol group, the 1- and 2-year cumulative survival rates were 80 and 60%, respectively. In the DSM group, they were 87 and 40%, respectively. In the lipiodol +DSM

group, they were 87 and 67%, respectively. No significant differences between the 3 groups were seen in survival (lipiodol group vs. DSM group, $P = 0.377$; lipiodol group vs. lipiodol + DSM group, $P = 0.560$; DSM group vs. lipiodol + DSM group, $P = 0.212$).

By the final follow-up, 21 patients remained alive (lipiodol group, $n = 8$; DSM group, $n = 6$; lipiodol + DSM group, $n = 7$), while the other 24 patients had died (lipiodol group, $n = 7$; DSM group, $n = 9$; lipiodol + DSM group, $n = 8$). In the lipiodol group, the cause of death was cancer progression in 6 patients and hepatic failure in 1 patient. In the DSM group, the cause of death was cancer progression in 7 patients, hepatic failure in 1 patient, and another disease in 1 patient. In the lipiodol + DSM group, the cause of death was cancer progression in 3 patients, hepatic failure in 2 patients, another disease in 2 patients, and rupture of esophageal varices in 1 patient.

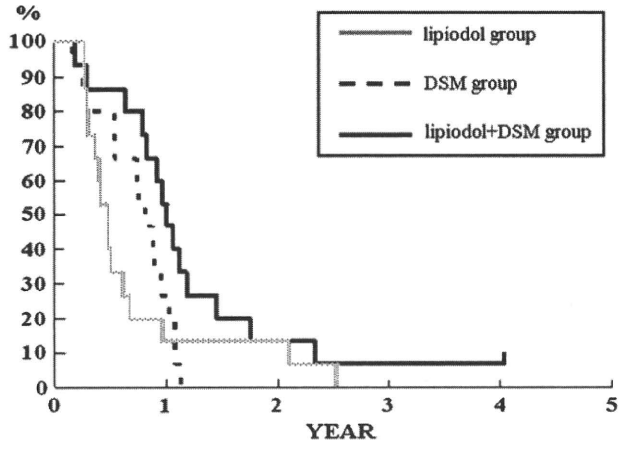

Fig. 2 Progression-free survival rates for the 3 groups. The 1- and 2-year progression-free survival rates in the lipiodol group were 13 and 13%, respectively. The 1-year progression-free survival rate was 27% in the DSM group. The 1-, 2-, and 3-year progression-free survival rates in the lipiodol + DSM group were 53, 13, and 7%, respectively. No significant difference in progression-free survival was seen between the lipiodol group and the DSM group ($P = 0.515$). On the other hand, progression-free survival in the lipiodol + DSM group was significantly better than that in the DSM group ($P = 0.020$) and the lipiodol group ($P = 0.035$)

Adverse effects of therapy

Table 3 shows the adverse effects of therapy. There was no significant difference in thrombocytopenia between the 3 groups, although grade 3 thrombocytopenia occurred in 4 patients of the lipiodol group (26.7%) and grade 3 or 4 thrombocytopenia occurred in 5 patients of the lipiodol + DSM group (33.3%). However, only 1 patient in the lipiodol + DSM group required a blood transfusion. The grade of elevated alanine aminotransferase (ALT) levels was significantly higher in the lipiodol + DSM group than in the lipiodol group ($P = 0.043$), although there were no significant differences in any other adverse effects between the 3 groups. No treatment-related deaths were observed in the 3 groups.

Figure 3 shows the changes in serum ALT or platelets before and after treatment in the lipiodol + DSM group. Transient increases in serum ALT concentration were observed in almost all patients; however, 2 weeks after treatment, concentrations decreased almost to pretreatment levels. Transient decreases in platelets were observed in almost all patients, and platelet counts at 3 days after treatment were the lowest before and after treatment; 2 weeks after treatment, the count increased almost to pretreatment levels.

Table 3 Adverse effects of therapy

Adverse effect	Lipiodol group ($n = 15$)/DSM group ($n = 15$)/lipiodol + DSM group ($n = 15$)				P value
	Grade 1	Grade 2	Grade 3	Grade 4	
Fever	12/5/8	0/1/0	0/0/0	0/0/0	NS
Nausea	0/2/1	0/0/1	0/0/0	0/0/0	NS
Appetite loss	2/5/2	0/0/0	0/0/0	0/0/0	NS
General fatigue	3/5/3	0/0/0	0/0/0	0/0/0	NS
Thrombocytopenia	3/0/0	4/5/5	4/1/3	0/0/2	NS
Creatinine	2/2/1	0/0/0	0/0/0	0/0/0	NS
ALT	12/5/5	3/5/6	0/3/3	0/0/0	0.043[#]
Diarrhea	0/0/1	0/0/0	0/0/0	0/0/0	NS
Ulcer	0/0/0	0/0/1	0/0/0	0/0/0	NS
Pleural effusion	0/0/0	0/0/1	0/0/0	0/0/0	NS
Pulmonary embolism	0/0/0	0/0/0	1/0/0	0/0/0	NS
Ascites	0/1/0	0/0/0	0/0/0	0/0/0	NS
Biloma	0/0/1	0/0/0	0/0/0	0/0/0	NS

According to Common Terminology Criteria for Adverse Events v. 4.0

DSM degradable microspheres, *ALT* alanine aminotransferase, *NS* not significant

[#] Lipiodol group versus lipiodol + DSM group

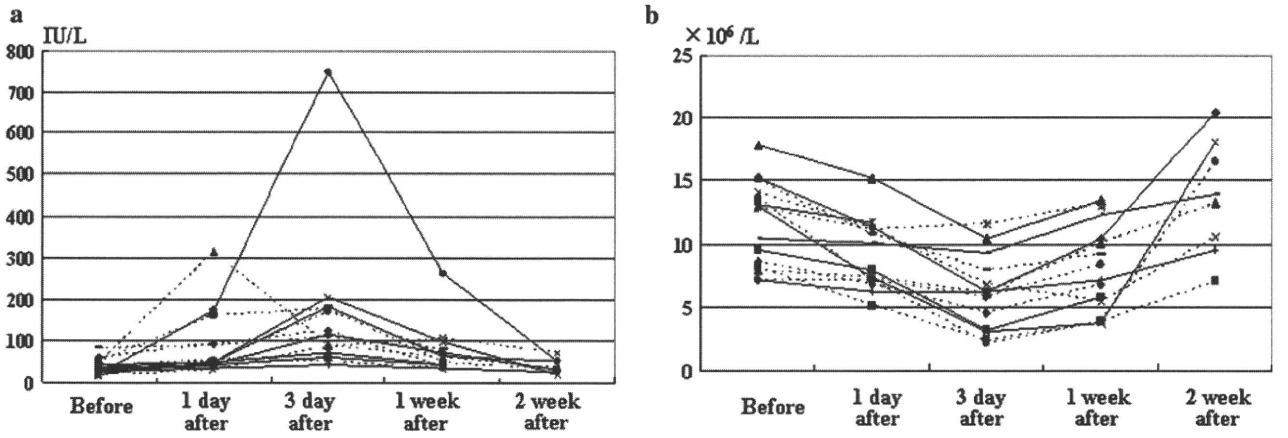

Fig. 3 Changes in serum alanine aminotransferase (ALT) (**a**) or platelet (**b**) levels before and after treatment in the lipiodol + DSM group. Transient increases in serum ALT concentration were observed in almost all patients; however, 2 weeks after treatment, the concentration decreased almost to pretreatment levels. Transient decreases in platelet levels were observed in almost all patients, and platelet counts at 3 days after treatment were lower than before and after treatment; 2 weeks after treatment, the count increased almost to pretreatment levels

Discussion

We designed a novel TAI using lipiodol and DSM for use in HCC patients, and reported the usefulness of this procedure [28]. In this study, we investigated the efficacy of this novel TAI using lipiodol and DSM in a prospective randomized trial (lipiodol vs. DSM vs. lipiodol + DSM).

We used a fine-powder formulation of cisplatin (IA-call; Nippon Kayaku Co., Tokyo, Japan) as the anticancer agent. The most common single-agent anticancer drug was doxorubicin, followed by cisplatin [12]. Although there is no evidence of the superiority of any chemotherapeutic agents [12], only a nonrandomized trial by Ono et al. [38] showed that cisplatin was better than doxorubicin. A Phase II study of hepatic arterial infusion of a fine-powder formulation of cisplatin reported that the response rate was 33.8% [39]. Therefore, we selected IA-call as the anticancer agent.

In our study, the response rates (patients with TE VI and III/all patients) in the lipiodol group, DSM group, and lipiodol + DSM group were 40, 53.4, and 80%, respectively. The CR rate (patients with TE IV/all patients) in particular was 40% in the lipiodol + DSM group. Although no significant differences between the 3 groups were seen due to the small population, the response rate tended to improve in the lipiodol + DSM group (lipiodol group vs. lipiodol + DSM group, $P = 0.07$; Mann–Whitney U test). Because of the response rate results, progression-free survival in the lipiodol + DSM group was significantly better than that in the DSM group ($P = 0.020$) and the lipiodol group ($P = 0.035$). On the other hand, no significant difference in progression-free survival was seen between the lipiodol group and the DSM group ($P = 0.515$).

Previous reports associated with our study are shown in Table 4. The response rate was 51% (CR rate 29%) in TAI using cisplatin and lipiodol [19]. On the other hand, the response rates were 73% (CR rate 32%) [19] and 45% (CR rate 0%) [38] in TACE using cisplatin and lipiodol. Although there is a difference in anticancer drugs, the response rates were 52.9% (CR rate 11.8%) [26] and 26% (CR rate 0%) [27] in TAI using DSM. The present findings showed that the response rates in the lipiodol group and in the DSM group were 40% (CR rate 26.7%) and 53.4% (CR rate 26.7%), respectively. Although it is difficult to compare the response rates of our data with those of previous reports, the response rates of the lipiodol group and the DSM group were similar to those of previous reports. On the other hand, only two clinical studies have evaluated TAI using lipiodol and DSM in HCC patients [40, 41]. However, there were some differences in embolization technique. Although the procedure of Vogl et al. [40] was similar to ours, the DSM dose was low (2–10 mg) compared with our procedure (60–1500 mg; mean, 426.6 ± 404.8 mg). Kirchhoff et al. [41] reported administering a mixture of anticancer drugs, DSM, and lipiodol and seeing a response rate of 36% (CR rate 0%). The particles of the emulsion using anticancer agents and lipiodol (lipiodol emulsion) are <30 μm [42] and those of DSM are 45 ± 7 μm in diameter [43]. Because the DSM particles are larger in diameter than those of the lipiodol emulsion, DSM may cause the occlusion of feeding tumor vessels before the accumulation of lipiodol emulsion by means of a mixture of DSM and lipiodol emulsion. In our study, the response rate was 80% (CR rate 40%). Our response rate is better than that reported by Kirchhoff et al. [41], and is similar to that of TACE reported by Ikeda et al. [19].

Table 4 Previous reports associated with our study

Author and reference	Embolizing agents	Anticancer drugs	Case no.	Response rate (CR rate)	Survival (%)
Ikeda [19]	Lipiodol	Cisplatin	94	51% (29%)	81.6/39.8 (1/3 year)
	Lipiodol, gelform	Cisplatin	74	73% (32%)	87.8/52.2 (1/3 year)
Fruse [26]	DSM	Epirubicin	17	52.9% (11.8%)	64.7/45.3 (1/2 year)
Kirchoff [27]	DSM	Cisplatin, doxorubicin	35	26% (0%)	57/31 (1/2 year)
Kirchoff [41]	Lipiodol, DSM	Cisplatin, doxorubicin	47	36% (0%)	75/59 (1/2 year)
	Lipiodol	Cisplatin	15	40% (26.7%)	80/60 (1/2 year)
Our study	DSM	Cisplatin	15	53.4% (26.7%)	87/40 (1/2 year)
	Lipiodol, DSM	Cisplatin	15	80% (40%)	87/67 (1/2 year)

DSM degradable microspheres

Both animal and clinical studies have reported that lipiodol injected into the hepatic artery occasionally appears in the portal veins through multiple arterioportal communications [44, 45], and that lipiodol can be used to temporarily embolize both the hepatic arteries and the portal veins. We speculate that lipiodol emulsion may be pushed out in the portal vein, the drainage vein of HCC, by DSM. Consequently, we may achieve as tight an interruption of blood supply as TACE for HCC.

There were no significant differences between the 3 groups in adverse effects other than the grade of elevated ALT levels. However, we consider that the high level of ALT in the lipiodol + DSM group reflects the effect of embolization. Transient increases in serum ALT concentration decreased almost to pretreatment levels 2 weeks after TAI using lipiodol and DSM. Because no serious adverse effects were seen in the lipiodol + DSM group, we consider TAI using lipiodol and DSM to be a safe treatment.

In conclusion, our developed TAI using lipiodol and DSM was superior to TAI using lipiodol only and TAI using DSM only because of improvements in therapeutic effects and progression-free survival. This procedure is both a safe and an effective therapy for HCC patients. TAI using lipiodol and DSM may be expected to serve as an alternative to TACE. Since our study examined only a small population, further investigations are necessary.

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Autologous bone marrow cell infusion therapy for liver cirrhosis patients

Shuji Terai · Isao Sakaida

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Abstract We developed a novel cell therapy, autologous bone marrow cell infusion (ABMi) therapy, using autologous bone marrow, for liver cirrhosis patients. Our study depends on the findings from basic studies that bone marrow cell infusion repairs liver fibrosis in the cirrhotic liver, and improves liver function and the survival rate. Beginning in November 2003, we started a clinical study and found that ABMi therapy was safe and effective for liver cirrhosis patients. Multicenter trials in Japan and Korea have also shown the effectiveness of ABMi therapy. In this review, we report the current status of ABMi therapy for liver cirrhosis patients.

Keywords ABMi therapy · Bone marrow cell · Liver cirrhosis · GFP/CCl4 model · Liver repair cell

Abbreviations

CCl4	Carbon tetrachloride
GFP	Green fluorescent protein
BMI	Bone marrow cell infusion
ABMi	Autologous bone marrow cell infusion
MMP	Matrix metalloproteinase
LC	Liver cirrhosis
MNC	Mononuclear cell
FACS	Fluorescent-activated cell sorter
LR cell	Liver repair cell

S. Terai (✉) · I. Sakaida
Department of Gastroenterology and Hepatology,
Yamaguchi University Graduate School of Medicine,
Minami-Kogushi 1-1-1, Ube, Yamaguchi 755-8505, Japan
e-mail: terais@yamaguchi-u.ac.jp

Introduction

We began a clinical trial of autologous bone marrow cell infusion (ABMi) therapy for liver cirrhosis (LC) patients in November 2003 [1]. We then conducted a multicenter trial in Japan, in collaboration with a Korean group [2]. We have now performed ABMi therapy in 26 LC patients in Japan. The safety and effectiveness of ABMi therapy has been confirmed. In this review, we report the basic findings for the development of ABMi therapy and the current status of the clinical study.

Basic study for development of ABMi therapy

Possible stem cells have been identified in human bone marrow (BM) [3, 4]. Thus, BM was considered to be a novel source of cells for liver regenerative therapy [5–7]. We subsequently developed a green fluorescent protein (GFP)/carbon tetrachloride (CCl4) model that monitors the dynamic state of injected GFP-positive bone marrow cells (BMCs) in CCl4-induced persistent liver damage [6]. In the GFP/CCl4 model, we found that infused BMC repopulated in the cirrhotic liver and improved the function of the cirrhotic liver (Fig. 1). The infused BMC expressed matrix metalloproteinase (MMP9) and migrated into damaged areas [7]. Finally, BMC infusion alleviated liver fibrosis and improved the liver microenvironment in cirrhotic mice [8]. In this step, fibroblast growth factor 2 (FGF2) is important for the repopulation of BMC in the cirrhotic liver and for the repair of the liver microenvironment [9, 10]. In BM, liver repair (LR) cells may exist that repair the cirrhotic liver. From our previous data, we speculated that the Liv-8-negative fraction might be candidate LR cells [11–13]. Now we are analyzing the characteristics of candidate LR cells in

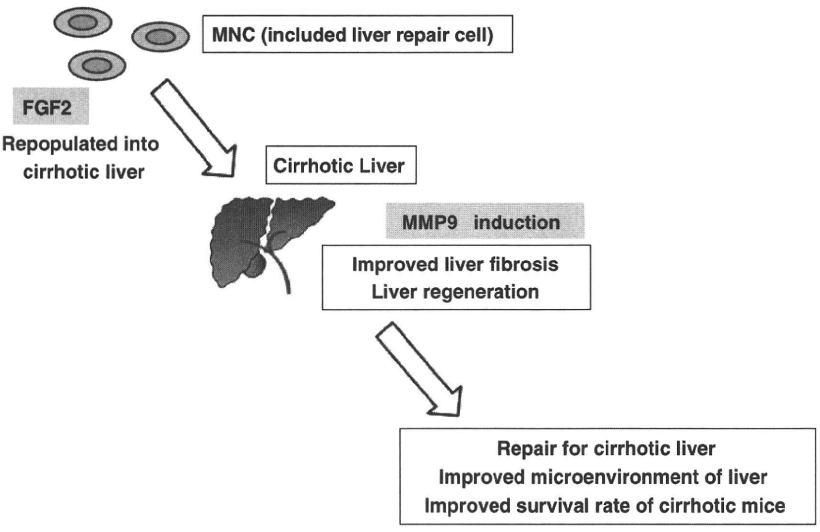

Fig. 1 Summary of the basic research. *FGF2*, Fibroblast growth factor 2; *MNC*, mononuclear cell; *MMP9*, matrix metalloproteinase 9

BM. The effects of stem cells on liver fibrosis were also analyzed by another group [14, 15]. Bone marrow cell (BMC) infusion and mesenchymal stem cell infusion were found to alleviate liver fibrosis in another mouse model. Based on these studies, BM cell infusion appears to improve the microenvironment in the cirrhotic liver [7, 8]. MMP9 expression in the cirrhotic liver was induced by BMC infusion. The MMP9 induction might be a key mechanism to understand the mechanism of repair of the cirrhotic liver. This repair mechanism is important for the development of ABMi therapy for LC patients, so we are analyzing the mechanism more precisely.

Clinical study: ABMi therapy for LC patients

We started a clinical trial of ABMi therapy for LC patients in November 2003. The subjects were LC patients with total bilirubin (TB) <3.0 mg/dL, platelets (Plt) >5 (10^{10}/L), and no viable hepatocellular carcinoma on diagnostic imaging. Autologous BM (400 mL) was isolated from the ilium under general anesthesia. Mononuclear cells (MNCs) were separated by cell washing and were infused via a peripheral vein. The protocol of ABMi therapy is shown in Fig. 2. It takes around 1 h to get BM from the ilium and around 3 h for cell processing. MNC characteristics were confirmed by fluorescence-activated cell sorter (FACS) analysis (CD34, CD45, c-kit). In the first report of our clinical study, we administered ABMi therapy in 9 LC patients [1]. From 400 mL of BM, we obtained $5.2 \pm 0.63 \times 10^9$ MNCs, and these were infused into LC patients. Liver function was monitored by blood examination for 24 weeks after ABMi therapy. We then monitored liver function using ultrasonography, computed tomography (CT), and laboratory tests. Significant improvements in serum albumin levels and total

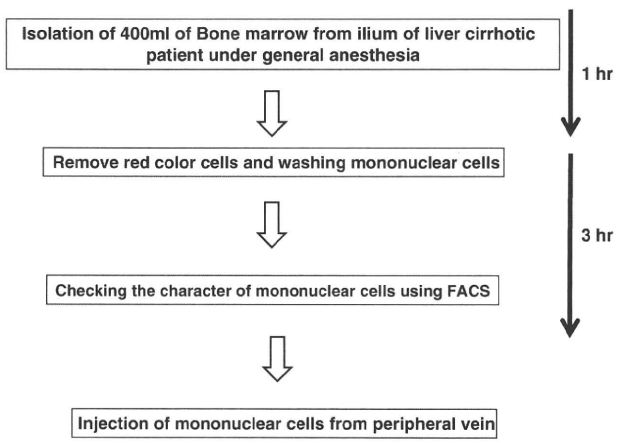

Fig. 2 The protocol for autologous bone marrow cell infusion (ABMi) therapy. *FACS*, Fluorescent-activated cell sorter

protein were seen at 24 weeks after ABMi therapy ($P < 0.05$). The Child-Pugh score had improved significantly at 4 and 24 weeks after ABMi therapy ($P < 0.05$). In addition, alpha fetoprotein (AFP) and proliferating cell nuclear antigen (PCNA) expression in liver biopsy tissue was significantly elevated after ABMi therapy ($P < 0.05$). These results suggested that ABMi therapy might induce repair of the cirrhotic liver [1]. No severe adverse effects were observed.

A multicenter trial of ABMi therapy in Japan was also carried out at Yamagata University, collaborating with Yamaguchi University, beginning in February 2006. At Yonsei University in Korea, the Yamaguchi-Yonsei collaboration study for ABMi therapy started in November 2006. In these clinical studies, the safety and effectiveness of ABMi therapy were also confirmed. In India and Brazil, cell therapy using BMCs for LC patient has also been studied, and its effectiveness has been confirmed [16].

These results suggested that LR cells in BM might be useful for the repair of the cirrhotic liver.

Future prospects

Based on previous clinical studies, we found that cell therapy using autologous BMCs was safe and effective for LC patients [1, 2]. Recently, in Iran, a similar study was performed and the effectiveness of cell therapy using BMCs was also confirmed [17, 18]. To develop more effective therapy and to cure patients with more severe LC, we are trying to identify the candidate LR cells in BM. As the next project, we intend to develop a method for the expansion of BM-derived LR cells and carry out new reparative cell therapy for LC patients.

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Original Article

Effect of a late evening snack using branched-chain amino acid-enriched nutrients in patients undergoing hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma

Yohei Harima, Takahiro Yamasaki, Satoe Hamabe, Issei Saeki, Kohsuke Okita, Shuji Terai and Isao Sakaida

Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, Ube, Yamaguchi, Japan

Aim: A late evening snack (LES) is recommended for protein-energy malnutrition in patients with liver cirrhosis. This study investigated energy metabolism in cirrhotic patients with hepatocellular carcinoma (HCC) and the effects of LES using a branched-chain amino acid (BCAA)-enriched nutrient in cirrhotic patients with advanced HCC undergoing hepatic arterial infusion chemotherapy (HAIC).

Methods: Energy metabolism was measured using indirect calorimetry for 10 cirrhotic patients without HCC and 36 patients with various stages of HCC. Next, in 23 cirrhotic patients with advanced HCC undergoing HAIC, 13 patients received LES (LES group), and 10 patients received ordinary food (control group). Changes in energy metabolism and glucose tolerance were examined using indirect calorimetry and 75-g oral glucose tolerance test (OGTT) before and after 1 cycle of treatment.

Results: Non-protein respiratory quotient (npRQ) was significantly lower in patients with advanced HCC than in cirrhotic patients without HCC, or in patients with early-stage HCC. In cirrhotic patients with advanced HCC undergoing HAIC, npRQ, BCAA/tyrosine ratio (BTR), and prealbumin and ALT levels were significantly improved in the LES group, but not in controls. In addition, area under the concentration curve for glucose (AUC glucose) tended to be improved in the LES group.

Conclusions: LES using BCAA-enriched nutrients appears to improve energy metabolism and glucose tolerance in cirrhotic patients with advanced HCC undergoing HAIC.

Key words: advanced hepatocellular carcinoma, branched-chain amino acid, hepatic arterial infusion chemotherapy, late evening snack, nutritional therapy

INTRODUCTION

THE LIVER PLAYS an important role in energy metabolism, and liver diseases lead to abnormalities in nutrient metabolism and subsequent malnutrition.[1] Protein-energy malnutrition (PEM) is a common finding in cirrhotic patients.[2,3] Owen *et al.* reported that patients with cirrhosis show marked decreases in glucose oxidation after an overnight fast, with enhanced fat and protein catabolism similar to that observed in healthy controls after 2–3 days of starvation.[4] PEM is a significant factor in establishing the vital prognosis of liver cirrhosis.[3]

In an attempt to improve the state of energy malnutrition, a late evening snack (LES) has been developed for use by patients with liver cirrhosis, resulting in improved energy substrate metabolism.[5–8] A LES is recommended in the present guidelines of the American Society for Parenteral and Enteral Nutrition[9] and the European Society for Clinical Nutrition and Metabolism.[10] We have also reported that a LES using branched-chain amino acid (BCAA)-enriched nutrients improves energy malnutrition, imbalances in amino acids, and

Correspondence: Dr Takahiro Yamasaki, Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minamikogushi, Ube, Yamaguchi 755-8505, Japan. Email: t.yama@yamaguchi-u.ac.jp
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glucose intolerance in patients with liver cirrhosis.[11–13] However, those studies focused on the effects of LES in patients with liver cirrhosis.

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer in the world.[14] Deaths due to HCC are increasing in almost all countries around the world, including Japan.[15–17] In particular, the prognosis for patients with advanced HCC showing portal vein tumor thrombosis (PVTT) remains poor.[18] Such patients are thus generally treated with hepatic arterial infusion chemotherapy (HAIC).[19–21] Our previous study identified Child-Pugh score[22] as an independent prognostic factor in cirrhotic patients with advanced HCC treated using HAIC.[20,21] In addition, energy expenditure in cirrhotic patients with HCC is reportedly increased compared with that in cirrhotic patient without HCC,[23] and a hypermetabolic rate in patients with gastrointestinal malignancy has been associated with the most advanced stage of the disease.[24]

Given this background, we consider that nutritional support is required for cirrhotic patients with advanced HCC undergoing HAIC. Few reports have examined nutritional support in cirrhotic patients with HCC.[25,26] Furthermore, no clinical studies have evaluated energy metabolism using indirect calorimetry in patients with HCC. We therefore investigated energy metabolism in patients with HCC and the efficacy of nutritional support using LES in patients with advanced HCC undergoing HAIC.

MATERIALS AND METHODS

Energy metabolism in patients with HCC

Patients

WE INVESTIGATED ENERGY metabolism using indirect calorimetry in cirrhotic patients without HCC and with various stages of HCC under the same conditions of liver capacity. Subjects comprised 10 cirrhotic patients without HCC and 36 patients with HCC before treatment ($n = 46$). No patients had received BCAA-enriched nutrients, and all were classified as Child-Pugh A.[22] Table 1 summarizes the clinical profiles of the 46 patients in this study. Tumor stage was determined according to the criteria of the Liver Cancer Study Group of Japan.[27,28] Liver cirrhosis was present in 10 patients, stage I/II HCC in 13 patients, stage III HCC in 13 patients, and stage IV HCC in 10 patients. The 4 groups showed no significant differences in clinical characteristics other than age (HCC stage III group vs. HCC stage IV group, $P = 0.017$). In addition, no significant differences in laboratory parameters, including BCAA/tyrosine ratio (BTR),[29] were identified among the 4 groups.

Energy metabolism was analyzed using indirect calorimetry (Deltatrac II; Detex Ohmeda, Helsinki, Finland). Indirect calorimetry was performed for 30 min after overnight bed rest and fasting. We measured oxygen consumption per minute (VO_2), carbon dioxide

Table 1 Clinical profiles of the 46 patients with and without hepatocellular carcinoma

Clinical characteristics	LC (n = 10)	HCC (n = 36)		
		Stage I/II† (n = 13)	Stage III† (n = 13)	Stage IV† (n = 10)
Age	66.9 ± 9.2	69.1 ± 8.0	73.8 ± 9.4*	62.2 ± 11.2*
Sex (male/female)	4/6	9/4	8/5	8/2
HCV Ab(+)/HBs Ag(+)/others	5/2/3	11/1/1	8/4/1	5/3/2
Child-Pugh A(5)/A(6)	6/4	8/5	10/3	7/3
Total Protein (g/dL)	7.20 ± 0.58	7.25 ± 0.76	7.45 ± 0.57	7.45 ± 0.58
Albumin (g/dL)	3.62 ± 0.46	3.78 ± 0.51	3.88 ± 0.25	3.66 ± 0.28
BTR	4.46 ± 1.16	4.52 ± 1.45	5.19 ± 0.96	4.86 ± 1.06
NH3	46.6 ± 11.7	45.6 ± 25.0	40.9 ± 16.9	59.6 ± 30.2
Total cholesterol	160.3 ± 30.1	166.5 ± 30.1	176.9 ± 35.1	159.8 ± 13.7
ChE	193.0 ± 73.6	214.9 ± 89.6	255.0 ± 81.4	204.8 ± 60.1
CHI	79.7 ± 16.7	66.2 ± 17.6	68.0 ± 13.4	78.6 ± 18.5
BMI	23.1 ± 1.8	21.4 ± 2.6	22.9 ± 4.8	23.7 ± 3.5

*$P = 0.017$.
†According to the criteria of the Liver Cancer Study Group of Japan.
BMI, body mass index; BTR, branched-chain amino acid/tyrosine ratio; ChE, cholinesterase; CHI, creatinine height index; HCC, hepatocellular carciunoma; HCV, hepatitis C virus; LC, liver cirrhosis; NH3, ammonia.

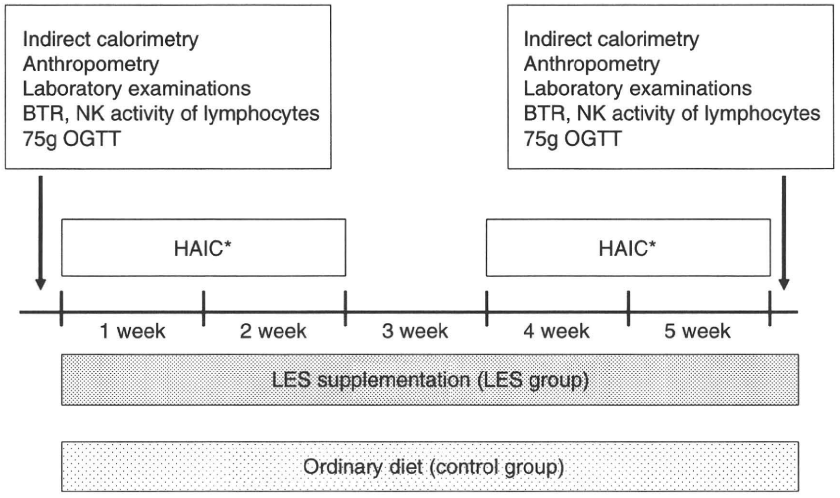

Figure 1 Study protocol. In the late evening snack (LES) group, patients received a LES supplement comprising branched-chain amino acid-enriched nutrients. In the control group, patients received ordinary food to the same amount of calories as the LES group. Before and after 1 cycle of hepatic arterial infusion chemotherapy (HAIC), nutritional evaluation using indirect calorimetry and InBody, laboratory examinations, and glucose tolerance using the 75-g oral glucose tolerance test were measured.

production per minute (VCO_2) and total urine nitrogen (TUN) on the day prior to examination, and the non-protein respiratory quotient (npRQ) was calculated as a measure of energy metabolism for the 4 groups.

Effect of LES for advanced HCC during HAIC

Patients

This study was performed on cirrhotic patients with unresectable HCC undergoing HAIC who had been admitted to the Department of Gastroenterology and Hepatology at Yamaguchi University Graduate School of Medicine. Eligibility criteria for this study were as follows: age, 20–80 years; Child-Pugh score A or B;[22] leukocyte count, ≥3000/mm^3; platelet count, ≥50 000/mm^3; serum creatinine, <1.2 mg/dL; unresectable HCC due to extensive, locally advanced disease that did not permit resection, bilobar disease, extrahepatic metastasis, or PVTT; and Eastern Cooperative Oncology Group (ECOG) performance status 0–2.[30]

Between December 2007 and February 2009, 26 patients were enrolled in this study. After randomization using a random number table, 13 patients received LES using a BCAA-enriched nutrient (LES group), and 13 received ordinary food (control group). However, 3 patients dropped out of the control group after withdrawing from the study during treatment. Thus, 13 patients in the LES group and 10 patients in the control group were subjected to analysis.

All patients provided written informed consent prior to enrolment into the study, and all protocols were approved by the Institutional Review Board of Yamaguchi University Hospital.

Table 2 summarizes the clinical profiles of patients in the 2 groups. No significant differences between groups were seen in clinical characteristics.

Table 2 Clinical profiles of the 23 patients with hepatocellular carcinoma

Clinical characteristics	LES group (n = 13)	Control group (n = 10)	*P*-value
Age	64.5 ± 9.5	66.4 ± 12.8	0.69
Sex (male/female)	11/2	8/2	0.78
HCV Ab(+)/HBs Ag(+)/others	7/5/1	8/1/1	0.35
Child-Pugh A/B	6/7	6/4	0.58
Maximum tumor size (mm)	77.7 ± 50.5	88.0 ± 39.7	0.60
Tumor stage II/III/IV A/IV B†	1/3/3/6	1/2/6/1	0.31
CHI	66.7 ± 16.3	72.0 ± 22.7	0.65
BMI	23.3 ± 3.9	21.4 ± 2.8	0.22

†According to the criteria of the Liver Cancer Study Group of Japan.
BMI, body mass index; CHI, creatinine height index; LES, late evening snack.

Study protocol

The intervention schedule is presented in Figure 1. Before this study, nutritional education was presented to all patients by dieticians. Daily nutritional intake for each group was calculated as 25–30 kcal with 1.2–1.3 g of protein per kilogram of ideal body weight per day. In the LES group, actual daily nutritional intake from meals was determined by subtracting the calorie content of LES (210 kcal) and protein (13.5 g) from the aforementioned calculated nutritional intake. One pack of the BCAA-enriched mixture (Aminoleban EN; Otsuka, Tokyo, Japan) used as LES food (at 22:00) contains 210 kcal of energy, 31.05 g of carbohydrate, 13.5 g of protein, 3.5 g of fat, and trace amounts of minerals and vitamins.[31] In the control group, patients received ordinary food with the same calorie content as the LES group.

After insertion of a 5-Fr heparin-coated catheter (Anthron P-U Catheter; Toray Medical, Tokyo, Japan) connected to a subcutaneously implanted reservoir, as described in a previous report,[19] patients received repeated arterial infusion of chemotherapeutic agents via the injection port.[20] One course of chemotherapy comprised 5 consecutive days of daily administration of cisplatin (10 mg/body/day on days 1–5; Randa; Nippon Kayaku, Tokyo, Japan) and isovorin (6.25 mg/body/day on days 1–5; Wyeth, Tokyo, Japan), followed by 5-fluorouracil (250 mg/body/day on days 1–5; Kyowa Hakko, Tokyo, Japan). Days 6 and 7 were rest days. This course was repeated for 2 weeks, followed by a 1-week suspension of chemotherapy. The course was then repeated for 2 weeks.

Nutritional parameters

Energy metabolism was analyzed by indirect calorimetry, and npRQ was calculated. A multi-frequency bioelectrical impedance analysis method (InBody 3.2; BIOSPACE, Tokyo, Japan) was used for anthropometric measurements.

Before and after 1 cycle of treatment, nutritional evaluation by indirect calorimetry and InBody, and changes in laboratory examinations, BTR, natural killer (NK) activity of lymphocytes,[32] plasma glucose and insulin level after 75-g oral glucose tolerance test (OGTT) were measured. The 75-g OGTT was performed at 4 time points: before administration, and at 30, 60 and 120 min. Area under the concentration curve for glucose (AUC glucose) and area under the concentration curve for insulin (AUC insulin) were determined using the above-mentioned 4 points and compared between before and after 1 cycle of treatment. We also divided patients into 3 groups according to blood glucose level 120 min after 75-g OGTT. A normal pattern (normal glucose tolerance [NGT]) was defined as blood glucose level <140 mg/mL at 120 min after 75-g OGTT. In comparison, a diabetic pattern (diabetes mellitus[DM]) was defined as glucose level >200 mg/mL and a borderline pattern (impaired glucose tolerance [IGT]) was defined as 140–200 mg/mL at 120 min after 75-g OGTT. CHI reflects skeletal muscle volume,[33] and was calculated using the following formula: CHI = (urinary creatinine excretion per day (mg))/(ideal body weight × A), where A is 23 for males and 18 for females.

Assessment of therapeutic efficacy

Dynamic computed tomography (CT) was performed before and after treatment. Tumor response was assessed on completion of 1 cycle of treatment. Response was classified according to ECOG criteria.[30] Complete response (CR) was defined as disappearance of all measurable lesions with no remaining signs, symptoms, or biochemical changes related to the tumor, which must have existed for >4 weeks, and appearance of no new lesions. Partial response (PR) was defined as a reduction of >50% in the sum of the products of the greatest perpendicular diameters of all measurable lesions, and appearance of no new lesions. Stable disease (SD) was defined as a reduction of <50% or an increase of <25% in the sum of the products of the greatest perpendicular diameters of all measurable lesions, and appearance of no new lesions. Progressive disease (PD) was defined as an increase of >25% in the sum of the products of the greatest perpendicular diameters of all measurable lesions, or appearance of new lesions.

Statistical analysis

Data are expressed as mean±standard deviation. Statistical analyses were performed using the unpaired *t*-test and the Mann-Whitney *U*-test, as appropriate. Survival period was calculated using the Kaplan-Meier method[34] from the date on which chemotherapy was started until death, and significance was determined by the log-rank test. Survival was confirmed up to 31 October, 2009. Values of $P < 0.05$ were considered statistically significant.

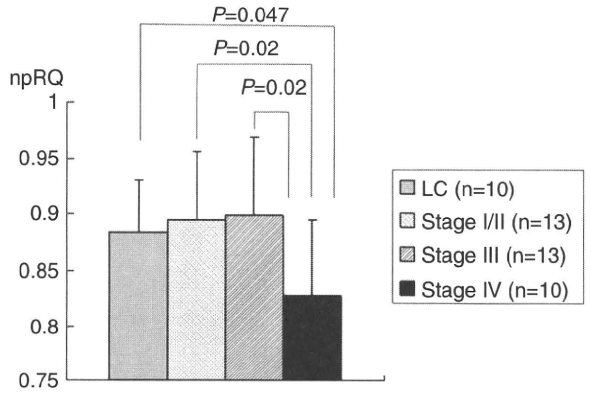

Figure 2 Value of non-protein respiratory quotient (npRQ) in cirrhotic patients without hepatocellular carcinoma (HCC) and with various stages of HCC. No significant difference in npRQ was seen among 3 groups (LC group, HCC stage I/II group, and HCC stage III group). However, npRQ was significantly lower in patients with stage IV HCC than in cirrhotic patients without HCC, or in patients with stage I/II or stage III HCC (LC group vs. HCC stage IV group, $P = 0.047$; HCC stage I/II group vs. HCC stage IV group, $P = 0.02$; HCC stage III group vs. HCC stage IV group, $P = 0.02$).

RESULTS

Energy metabolism in patients with HCC

FIGURE 2 SHOWS npRQ in cirrhotic patients without HCC and with various stages of HCC. Values of npRQ in cirrhotic patients without HCC, with stage I/II HCC, with stage III HCC, and with stage IV HCC were 0.88 ± 0.05, 0.89 ± 0.06, 0.90 ± 0.07, and 0.83 ± 0.07, respectively. No significant differences in npRQ were identified among the 3 groups (LC group, HCC stage I/II group, and HCC stage III group). However, npRQ was significantly lower in patients with stage IV HCC than in cirrhotic patients without HCC, or in patients with stage I/II or stage III HCC (LC group vs. HCC stage IV group, $P = 0.047$; HCC stage I/II group vs. HCC stage IV group, $P = 0.02$; HCC stage III group vs. HCC stage IV group, $P = 0.02$).

Effect of LES for advanced HCC during HAIC

Response to therapy

In the LES group ($n = 13$), 0 (0%), 3 (23%), 8 (62%), and 2 (15%) patients exhibited CR, PR, SD, and PD, respectively (response rate [patients with CR+PR/all patients], 23%). In the control group ($n = 10$), 1 (10%), 2 (20%), 4 (40%), and 3 (30%) patients exhibited CR, PR, SD, and PD, respectively (response rate, 30%). No significant differences in response rates were seen between groups ($P = 0.90$; Mann-Whitney *U*-test). As a result, no significant differences between groups were seen in relation to background.

Energy metabolism

Table 3 shows changes in npRQ before and after 1 cycle of treatment. The value of npRQ increased significantly after 1 cycle of treatment in the LES group (0.81 ± 0.08 vs. 0.88 ± 0.08, $P = 0.01$). However, npRQ did not differ in the control group (0.85 ± 0.08 vs. 0.86 ± 0.06,

Table 3 Changes in energy metabolism and laboratory parameters

	LES group (n = 13)			Control group (n = 10)		
	Before	After	*P*-value	Before	After	*P*-value
npRQ	0.81 ± 0.08	0.88 ± 0.08	0.01	0.85 ± 0.08	0.86 ± 0.06	0.69
BTR	3.76 ± 0.90	4.55 ± 1.38	0.008	4.16 ± 1.03	3.97 ± 1.22	0.47
Total Protein (g/dL)	7.25 ± 0.75	7.46 ± 0.97	0.29	7.32 ± 0.68	7.01 ± 0.59	0.17
Albumin (g/dL)	3.06 ± 0.51	3.08 ± 0.53	0.70	3.26 ± 0.48	3.24 ± 0.42	0.83
Prealbumin (mg/dL)	8.64 ± 4.49	10.17 ± 4.56	0.049	10.42 ± 4.76	11.43 ± 5.24	0.27
Total bilirubin (mg/dL)	0.98 ± 0.55	1.05 ± 0.75	0.57	1.05 ± 0.35	1.00 ± 0.37	0.75
ALT (IU/L)	38.6 ± 31.3	31.3 ± 12.9	0.04	50.9 ± 35.14	36.9 ± 23.22	0.67
PT (%)	76.0 ± 12.5	76.9 ± 7.9	0.72	81.5 ± 9.86	82.6 ± 10.8	0.76
Total cholesterol (mg/dL)	153.2 ± 31.1	153.2 ± 31.1	0.79	162.0 ± 57.4	167.6 ± 68.4	0.58
ChE (IU/L)	140.2 ± 70.4	134.2 ± 73.1	0.20	163.7 ± 68.6	131.1 ± 52.2	0.01
NH3 (μ mol/dL)	58.5 ± 23.1	69.3 ± 27.6	0.07	57.3 ± 23.2	66.0 ± 35.0	0.26
Natural killer cell activity (%)	24.8 ± 11.9	18.2 ± 13.8	0.14	25.5 ± 12.8	18.1 ± 10.9	0.16

ALT, alanine aminotransferase; BTR, branched-chain amino acid/tyrosine ratio; ChE, cholinesterase; LES, late evening snack; NH3, ammonia; PT, prothrombin time; npRQ, non-protein respiratory quotient.