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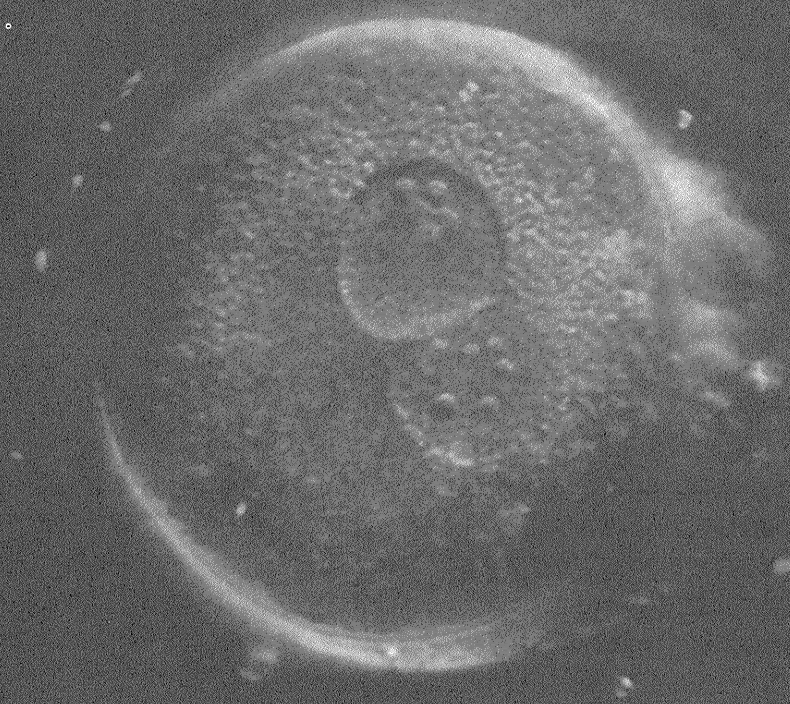

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

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

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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

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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.

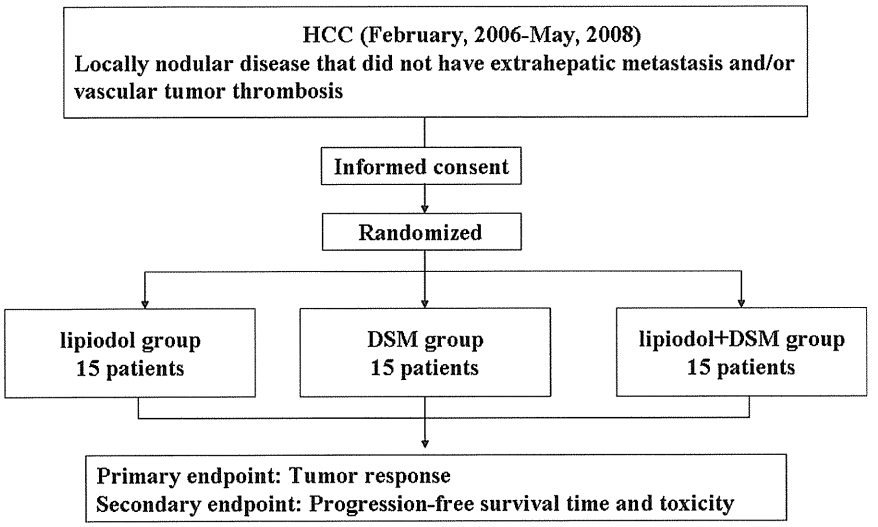

Fig. 1 Study design. We randomly divided patients into 3 groups: transcatheter arterial infusion chemotherapy (TAI) using lipiodol (lipiodol group, $n = 15$), TAI using degradable starch microspheres (DSM) (DSM group, $n = 15$), and TAI using lipiodol and DSM (lipiodol + DSM group, $n = 15$)

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.

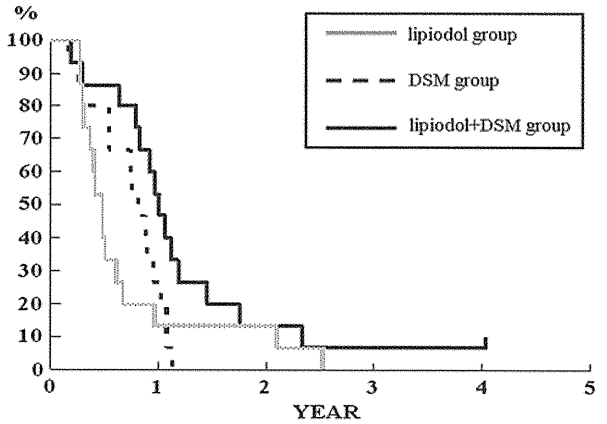

Fig. 2 Progression-free survival rates for the 3 groups. The 1- and 2-year progression-free survival rates in the lipiodol group were 13 and 13%, respectively. The 1-year progression-free survival rate was 27% in the DSM group. The 1-, 2-, and 3-year progression-free survival rates in the lipiodol + 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

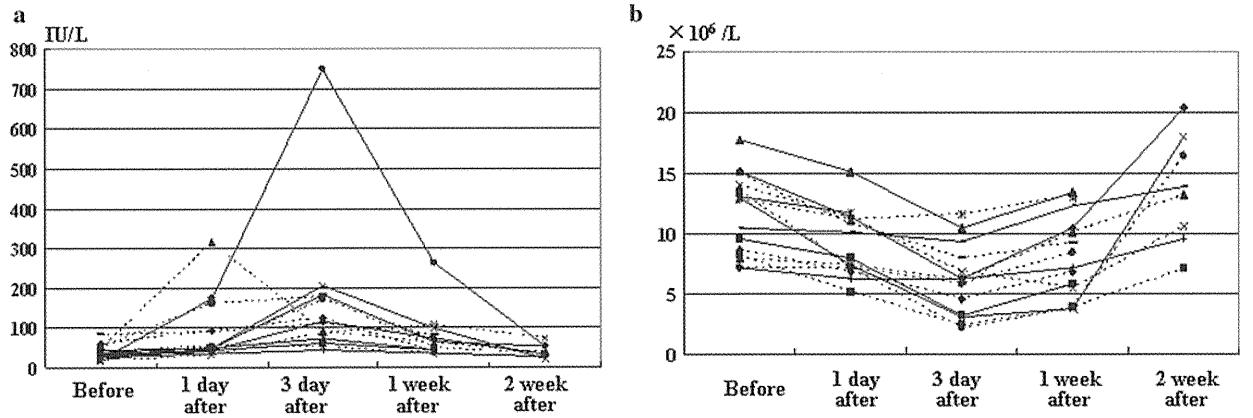

Fig. 3 Changes in serum alanine aminotransferase (ALT) (**a**) or platelet (**b**) levels before and after treatment in the lipiodol + DSM group. Transient increases in serum ALT concentration were observed in almost all patients; however, 2 weeks after treatment, the concentration decreased almost to 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)
Our study	Lipiodol	Cisplatin	15	40% (26.7%)	80/60 (1/2 year)
	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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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

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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

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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.

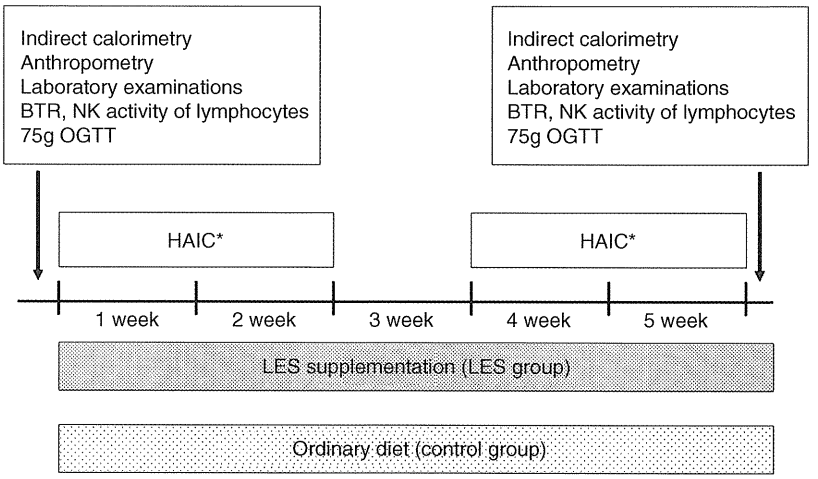

Figure 1 Study protocol. In the late evening snack (LES) group, patients received a LES supplement comprising branched-chain amino acid-enriched nutrients. In the control group, patients received ordinary food to the same amount of calories as the LES group. Before and after 1 cycle of hepatic arterial 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 PVIT; 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.

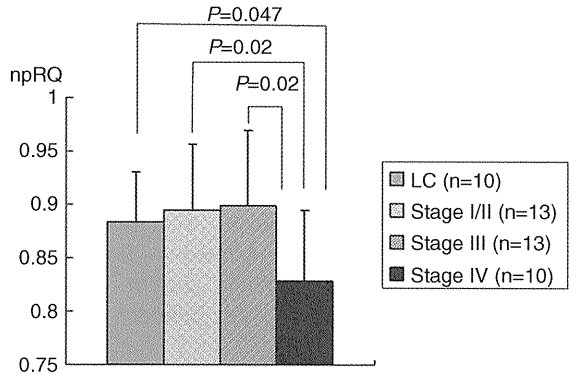

Figure 2 Value of non-protein respiratory quotient (npRQ) in cirrhotic patients without hepatocellular carcinoma (HCC) and with various stages of HCC. No significant difference in npRQ was seen among 3 groups (LC group, HCC stage I/II group, and HCC stage III group). However, npRQ was significantly lower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

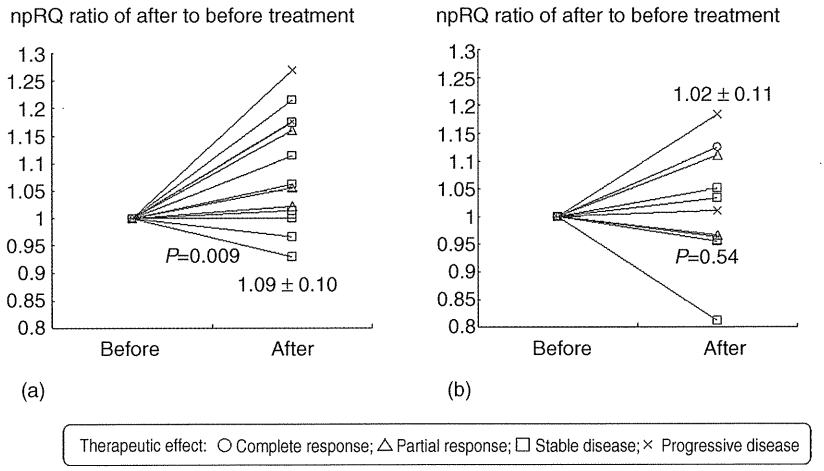

Figure 3 The npRQ ratio after compared to before 1 cycle of HAIC. In the LES group, npRQ improved in 10 patients, was stable in 1 patient, and worsened in 2 patients, regardless of response to therapy ($P = 0.009$) (a). In the control group, npRQ improved in 6 patients and worsened in 4 patients ($P = 0.54$) (b).

$P = 0.69$). Figure 3 shows the npRQ ratio of after compared to before 1 cycle of HAIC. In the LES group, npRQ improved in 10 patients, was stable in 1 patient, and worsened in 2 patients, regardless of response to therapy ($P = 0.009$). In the control group, npRQ improved in 6 patients and worsened in 4 patients ($P = 0.54$).

Blood biochemistry

Significant improvements in BTR, prealbumin and ALT levels were observed after 1 cycle of treatment in the LES group, but not in the control group. Cholinesterase levels were significantly decreased after 1 cycle of treatment in the control group, but did not differ in the LES group (Table 3). Figure 4 shows the BTR ratio of after to before 1 cycle of HAIC. In the LES group, BTR improved in 10 patients and worsened in 3 patients ($P = 0.005$). Conversely, BTR worsened in 7 patients in the control group ($P = 0.46$).

Anthropometry

No significant differences in anthropometric measurements (weight; skeletal muscle mass; body fat mass; fat-free mass; mid-upper arm muscle circumference (AMC); midarm circumference (AC); and body cell mass (BCM)) as measured using InBody were observed between groups (data not shown).

Changes in glucose tolerance

We examined the effects of LES using a BCAA-enriched nutrient on glucose tolerance using the 75-g OGTT in 21

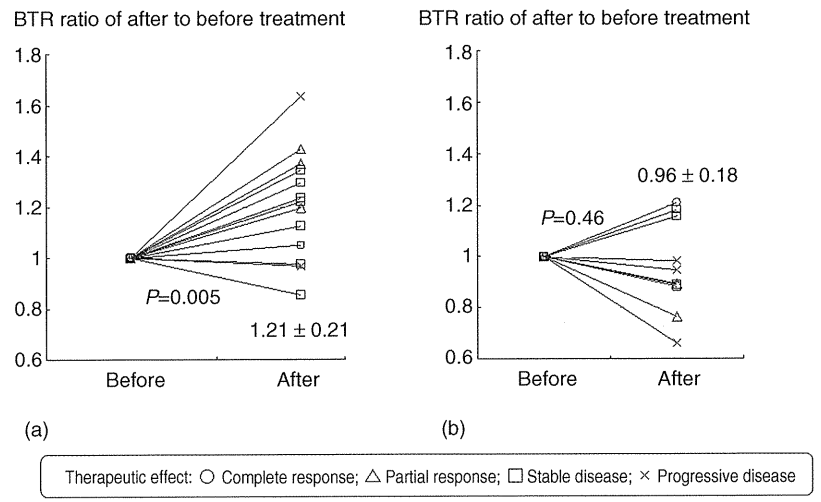

Figure 4 Branched-chain amino acid/tyrosine ratio (BTR) after compared to before 1 cycle of HAIC. In the LES group, BTR improved in 10 patients and worsened in 3 patients ($P = 0.005$) (a). Conversely, BTR worsened in 7 patients in the control group ($P = 0.46$) (b).

Table 4 Changes in glucose tolerance

	LES group (n = 12)			Control group (n = 9)		
	Before	After	*P*-value	Before	After	*P*-value
AUC glucose	396.1 ± 117.0	363.2 ± 135.6	0.055	355.3 ± 62.9	321.1 ± 108.6	0.17
AUC insulin	181.8 ± 123.3	223.2 ± 169.0	0.10	155.4 ± 85.3	117.7 ± 71.4	0.15
Fasting glucose (mg/dL)	99.5 ± 27.9	98.5 ± 32.6	0.71	100.7 ± 13.9	99.6 ± 18.7	0.89
Fasting insulin (μ U/mL)	11.3 ± 10.2	12.8 ± 7.1	0.38	14.5 ± 15.6	8.2 ± 1.8	0.28
HOMA-IR	2.9 ± 2.7	3.3 ± 2.5	0.47	4.0 ± 5.2	2.1 ± 0.8	0.32

AUC, area under the concentration curve; HOMA-IR, homeostasis model assessment method for insulin resistance; LES, late evening snack.

of 23 patients. One patient with DM in the LES group did not undergo the 75-g OGTT after treatment due to markedly high glucose levels, while the remaining patient in the control group declined to undergo the 75-g OGTT after treatment.

Table 4 shows changes in glucose tolerance before and after 1 cycle of treatment. In the LES group ($n = 12$), 1, 2, and 9 patients exhibited NGT, IGT, and DM, respectively. In the control group, 3, 2, and 4 patients exhibited NGT, IGT, and DM, respectively. No significant differences at baseline were seen between groups with regard to NGT, IGT, or DM using the 75-g OGTT, fasting glucose, fasting insulin, homeostasis model assessment method for insulin resistance (HOMA-IR), AUC glucose, and AUC insulin. AUC glucose tended to improve after 1 cycle of treatment in the LES group ($P = 0.055$). However, no significant differences in other parameters were apparent.

Prognosis

No significant differences in survival rates were seen between groups ($P = 0.667$; log-rank test) (Fig. 5a). On the other hand, survival in patients assessed as SD or PD according to the response criteria[30] tended to improve in the LES group ($n = 10$ in the LES group, $n = 7$ in the control group; $P = 0.156$; log-rank test) (Fig. 5b). In addition, no significant differences between groups assessed as SD or PD were seen with relation to background (data not shown).

By final follow-up, 2 patients remained alive (LES group, $n = 1$; control group, $n = 1$), while the other 21 patients had died. In the LES group, cause of death was cancer progression in 12 patients. In the control group, cause of death was cancer progression in 8 patients and hepatic failure in 1 patient.

Case presentation

Figure 6 shows a patient from the LES group. This 49-year-old man showed multiple HCCs in both lobes (stage III).[27,28] Mild ascites was identified, but no hepatic encephalopathy was present. On admission, hepatic reserve function was defined as Child-Pugh B (9 points). Prior to starting LES, npRQ was 0.71, and BTR value was low, at 2.2. After 1 cycle of HAIC, laboratory investigations were improved, and no ascites was apparent. Hepatic reserve function had improved to Child-Pugh A (6 points). Values of npRQ and BTR increased to 0.75 and 2.68, respectively, after 1 cycle of treatment. The patient exhibited DM on the 75-g OGTT. AUC glucose improved after 1 cycle of treatment (before, 414.75;

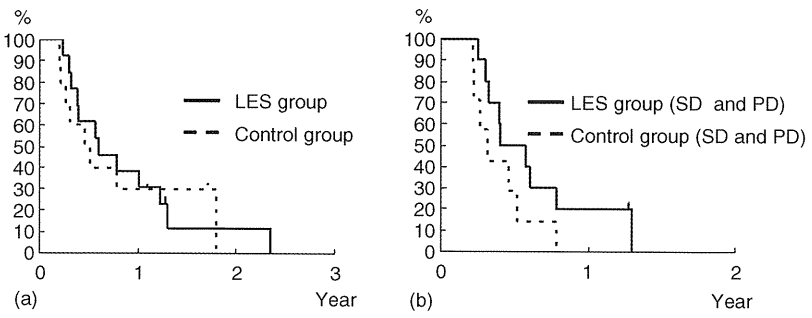

Figure 5 No significant differences in survival rates were seen between groups ($P = 0.667$; log-rank test) (a). On the other hand, survival in patients assessed as stable disease (SD) or progressive disease (PD) according to the response criteria tended to improve in the LES group ($n = 10$ in the LES group, $n = 7$ in the control group; $P = 0.156$; log-rank test) (b).

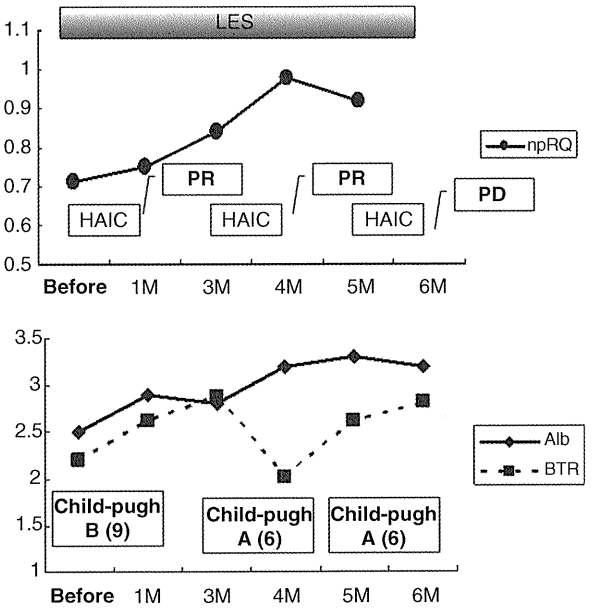

Figure 6 A case in the LES group. This 49-year-old man had multiple HCCs in both lobes (stage III). On admission, hepatic reserve function was Child-Pugh B (9 points). Prior to starting LES, npRQ was 0.71, and BTR was low at 2.2. After 1 cycle of HAIC, hepatic reserve function was improved to Child-Pugh A (6 points) from Child-Pugh B (9 points). Values of npRQ and BTR increased to 0.75 and 2.68, respectively, after 1 cycle of the treatment. The patient exhibited PR according to the response criteria. Thereafter, he received 3 courses of HAIC, and npRQ, serum albumin and BTR values improved. After the third course of HAIC, he exhibited PD. One year after the first course of HAIC, he died of tumor progression.

after, 369.75). PR was exhibited according to the response criteria.[30] Thereafter, the patient received 3 courses of HAIC, and npRQ, serum albumin and BTR value improved. After the third course of HAIC, he exhibited PD. One year after the first course of HAIC, he died of tumor progression.

Figure 7 shows a patient from the control group. This 73-year-old woman presented with massive HCC in the right lobe with tumor thrombus in the main trunk of the portal vein (Vp4) (stage IV A).[27,28] No ascites or hepatic encephalopathy was identified. On admission, hepatic reserve function was defined as Child-Pugh A (6 points). Prior to starting HAIC, npRQ was 0.91. However, BTR was low, at 2.34. After 1 cycle of HAIC, laboratory investigations showed slight deterioration. Values for npRQ and BTR decreased to 0.87 and 2.06, respectively, after 1 treatment cycle. The patient exhibited DM on the 75-g OGTT. AUC glucose remained almost unchanged (before, 446.25; after, 437.75). She exhibited SD according to the response criteria.[30] Although she received a second course of HAIC, npRQ, serum albumin and BTR values worsened. In addition, she showed moderate ascites during the second course of treatment. After the second course of treatment, hepatic reserve function was classified as Child-Pugh C (10 points). Thereafter, she developed hepatic encephalopathy and died of hepatic failure 6 months after the first course of HAIC.

DISCUSSION

PEM IS OFTEN observed in cirrhotic patients,[2,3] and this malnutrition adversely affects prognosis.[3] When energy metabolism of cirrhotic patients is measured using indirect calorimetry, npRQ decreases as the severity of liver cirrhosis increases.[3] However, no clinical studies have evaluated energy metabolism in patients with HCC. We therefore investigated energy metabolism

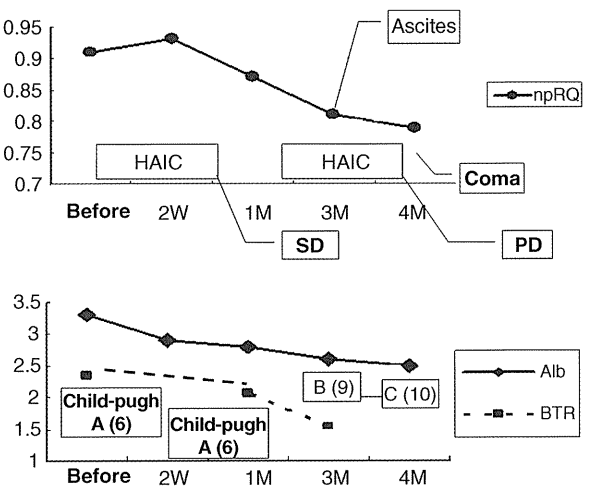

Figure 7 A case in the control group. This 73-year-old woman presented with massive HCC in the right lobe with tumor thrombus in the main trunk of the portal vein (Vp4) (stage IV A). On admission, hepatic reserve function was Child-Pugh A (6 points). Prior to starting HAIC, npRQ was 0.91. However, BTR was low at 2.34. After 1 cycle of HAIC, npRQ and the BTR value decreased to 0.87 and 2.06, respectively. She exhibited SD according to the response criteria. Although she received a second course of HAIC, npRQ, serum albumin and BTR values all worsened. After the second course of treatment, hepatic reserve function was Child-Pugh C (10 points). Thereafter, she developed hepatic encephalopathy and died of hepatic failure 6 months after the first course of HAIC.

using indirect calorimetry in cirrhotic patients without HCC and with various stages of HCC under the same conditions of liver capacity, namely Child-Pugh A. In our study, no significant differences were seen among 3 groups (LC group, HCC stage I/II group, and HCC stage III group), but 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. Although there was a significant difference in age between the HCC stage III group and the HCC stage IV group, there was no significant correlation between the value of npRQ before treatment and age (data not shown). These findings suggest that patients with advanced HCC remained under conditions of severe energy malnutrition even if liver capacity was good. A hypermetabolic rate in patients with gastrointestinal malignancy is reportedly usually associated with the most advanced stage of the disease.[24] The p53 tumor suppressor gene regulates glucose metabolism, and loss of p53 upregulates energy metabolism.[35] Mutation of the p53 gene is associated with poor tumor differentiation and advanced stage of HCC.[36] We speculate that reductions in npRQ among patients with advanced HCC may be related to the upregulation of glucose metabolism in cancer cells, but the underlying mechanisms remain unclear. Our results suggest that nutritional support is warranted for cirrhotic patients with advanced HCC, even if liver capacity is good.

In an attempt to improve the state of energy malnutrition, LES has been developed and improved energy metabolism has been reported.[5–8,11–13] However, those studies focused on the effects of LES in patients with cirrhosis.

Poon *et al.* reported that nutritional supplementation with oral BCAAs is beneficial for increasing serum albumin level, reducing morbidity and improving quality of life in patients undergoing transarterial chemoembolization for HCC,[25] but the nutritional supplementation did not use LES. Takeshita *et al.* only reported that LES using BCAA-enriched nutrients prevents suppression of liver function in patients with HCC undergoing transarterial chemoembolization.[26] That study did not evaluate energy metabolism before and after LES. We therefore investigated the efficacy of nutritional support using LES in patients with advanced HCC undergoing HAIC using indirect calorimetry.

The present findings showed that LES using BCAA-enriched nutrients improves npRQ, BTR, ALT, and prealbumin significantly before and after 1 cycle of HAIC compared with the control group. Nakaya *et al.* reported that LES using BCAA-enriched nutrients improved npRQ, BTR, and serum albumin before and 3 months after in cirrhotic patients compared with LES using ordinary food.[8] Although no significant difference in serum albumin was identified in our study, prealbumin (a rapid turnover protein with a half-life in plasma of 2 days) was significantly increased by LES. Prealbumin is more sensitive to changes in protein-energy status than albumin.[37] We thus consider that improvement of npRQ and prealbumin reflects energy metabolism in cirrhotic patients with advanced HCC undergoing HAIC. Unfortunately, we could not evaluate nutritional parameters in the long term, as some patients died in the short term. Despite the small sample size, of the 12 patients for whom npRQ was evaluated at 3 months after HAIC, patients treated with LES ($n = 6$) tended to show improved npRQ ($P = 0.10$), and npRQ was not significantly difference before and 3 months after HAIC in control patients ($n = 6$; $P = 0.91$; data not shown).

BCAAs reportedly improve glucose intolerance.[11–13,38] The present study evaluated glucose tolerance before and at the end of 1 cycle of treatment. AUC glucose in the LES group tended to improve ($P = 0.055$). In addition, AUC glucose in patients who showed glucose intolerance (IGT and DM; $n = 11$) in the LES group tended to improve (before, 412.3 ± 107.7; after, 376.1 ± 134.3; $P = 0.052$) and AUC glucose in patients who had glucose intolerance ($n = 6$) in the control group showed no significant difference (before, 376.5 ± 67.8; after, 338.3 ± 132.7; $P = 0.30$). One reason might be the effect of LES itself. A LES improves postprandial hyperglycemia, because the glucose load per meal is decreased by fractionated meals including a LES, and glucose is properly oxidized in the tissues. Another reason might be the effects of the leucine and isoleucine contained among the BCAAs. Leucine and isoleucine promote glucose uptake in skeletal muscle under insulin-free conditions.[39] Leucine also increases the activity of p70S6 kinase via the mammalian target of rapamycine pathway, and the ability to synthesize glycogen is improved.[39] However, we have previously reported that glucose tolerance worsened after 3 months of LES administration in cirrhotic patients with DM according to the 75-g OGTT.[40] In 12 patients (LES group, $n = 6$; control group, $n = 6$) for whom glucose tolerance was evaluated using the 75-g OGTT at 3 months after HAIC, AUC glucose was significantly worsened in both groups in this study (data not shown). We reported that LES combined with an alpha-glucosidase inhibitor to slow glucose absorption into the blood and ameliorate postprandial hyperglycemia improved glucose tolerance (AUC glucose) over the long term (3 months) in

patients with liver cirrhosis,[41] and concomitant use of an alpha-glucosidase inhibitor with LES might be a useful nutritional therapy in patients with advanced HCC who show glucose intolerance.

Unfortunately, no significant differences in survival rate were identified between groups ($P = 0.667$; log-rank test). Poon *et al.* also reported no difference in survival between patients who received BCAAs and those receiving ordinary food.[25] However, survival in patients assessed as SD or PD tended to improve in the LES group ($P = 0.156$; log-rank test), although no significant differences between groups assessed as SD or PD were seen with relation to background. Significant improvement in npRQ was observed in the LES group, and significant reductions in cholinesterase and natural killer cell activity were observed in the control group, for groups assessed as SD or PD (data not shown). In addition, the frequency of HCC treatment tended to be increased in the LES group (data not shown). We speculate that life prolongation in patients assessed as SD or PD may be related to improvements in energy metabolism and immune defense,[32] and the continuation of HCC treatment, by means of LES using BCAA-enriched nutrients. Our previous study identified therapeutic effect as an independent prognostic factor in cirrhotic patients with advanced HCC treated using HAIC.[20] In this study, patients exhibited CR or PR showed good prognosis without relation to LES (data not shown). Therefore, we consider that patients exhibited SD or PD may be suitable candidates for LES using BCAA-enriched nutrients because of life prolongation. As our study examined only a small population, further investigations are necessary.

In conclusion, LES using BCAA-enriched nutrients offers the possibility of improving energy metabolism and glucose tolerance in cirrhotic patients with advanced HCC undergoing HAIC. Although our study design shows limitations in the comparison between ordinary food and both LES and BCAA, we speculate that these results are caused by effects from both LES and BCAA. We consider that tailored nutritional support, such as tumor staging, is required in patients with HCC.

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