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Original Article

Recent progress in the management of hepatocellular carcinoma detected during a surveillance program in Japan

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Aim: This study explored recent improvements in the management of hepatocellular carcinoma (HCC) diagnosed during surveillance.

Methods: The subjects were 1074 patients with HCC, subdivided into three groups. Group A comprised 211 patients for whom HCC was detected during periodic follow-up examinations at Kurume University School of Medicine, Group B comprised 544 patients diagnosed with HCC during periodic follow-up examinations at other institutions, and, Group C comprised 319 patients with HCC detected incidentally or because of symptoms.

Results: In 1995–2000 and 2001–2006, 91% and 91% of group A, 68% and 70% of group B, and 27% and 26% of group C patients with HCC, respectively, met the Milan criteria. For groups A and B, the proportions of patients with Child–Pugh class A and use of promising treatment increased in the later

periods compared to those diagnosed during the earlier periods (group A, Child–Pugh class A, 72% vs 58% [$P = 0.040$], receiving treatment, 90% vs 70% [$P < 0.0001$]; group B, Child–Pugh class A, 71% vs 62% [$P = 0.031$]; receiving treatment, 72% vs 52% [$P < 0.0001$], respectively). The cumulative survival rates of the 405 patients with HCC detected in the latter 6 years tended to be better than those for patients diagnosed in the former 6 years (350 patients) (4 years, 58% vs 50% [$P = 0.0349$]).

Conclusion: The use of promising treatment and prognosis have improved in the last 6 years for patients with HCC diagnosed through surveillance relative to those identified in 1995–2000.

Key words: carcinoma, cirrhosis, hepatocellular surveillance, prognosis.

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common malignancies worldwide¹ and is the leading cause of death in patients with cirrhosis.² HCC commonly occurs in patients with chronic liver diseases related to hepatitis C virus (HCV) or hepatitis B virus (HBV) infection, with a reported incidence of HCC in patients with HCV of 1–8% per annum.^{3–7} Several cohort studies have shown that surveillance by abdominal ultrasonography (US) and α -fetoprotein (AFP) assay for patients with cirrhosis can detect early-stage HCC and thus have the potential to reduce mortality.^{4–13}

However, the results of surveillance are controversial including cost effectiveness^{14,15} due to the high annual incidence of HCC, the target population and frequency of surveillance, available treatment for HCC, management of cirrhosis, and possibly the US equipment and skill of the US examiner.

The advent of new imaging techniques for tumor staging and improved criteria for selection of patients for liver transplantation (LT), hepatic resection (HR) and locoregional ablative therapies (LAT) has improved survival rates in patients with HCC.^{2,16–23} Based on recent technological improvements in LAT, radiofrequency ablation therapy (RFA) has become more effective than percutaneous ethanol injection therapy (PEI) for patients with early-stage HCC.^{22,23} Moreover, recent progress in managing complications related to cirrhosis has prolonged the life of many patients with cirrhosis.^{2,24,25} These factors have contributed to the reported increase in survival of cirrhotic patients with HCC detected during surveillance over the three quinquennia (1987–2001).⁷

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We reported previously that surveillance for HCC at our Department of Liver Disease, Kurume University School of Medicine, successfully detected early-stage HCC, allowing a better chance of receiving promising treatment in 574 patients diagnosed from 1995–2001.¹³ In addition, Kurume University School of Medicine recently introduced RFA for the treatment of early-stage HCC,^{22,23} interferon (IFN) therapy for patients with cirrhosis, follow up after curative treatment of HCC^{26,27} and management of cirrhosis-related complications by nutritional therapists.^{24,25}

The present study explored the effects of recent improvements in managing HCC diagnosed through surveillance in Japanese hospitals.

METHODS

Patients

THE STUDY COMPRISED 1074 Japanese patients with HCC diagnosed at Kurume University School of Medicine from January 1995 to December 2006. The diagnosis of HCC was established by histopathology and/or imaging studies (US, computed tomography [CT], angiography, CT angiography, and magnetic resonance imaging [MRI]), and/or on high plasma levels of tumor markers such as AFP, lens culinaris agglutinin reactive AFP (AFP-L3), and des- γ -carboxy prothrombin (DCP). Patients were subdivided into three groups according to the manner of HCC detection: group A, 211 patients found to have HCC during periodic follow-up examination at Kurume University School of Medicine; group B, 544 patients found to have HCC during periodic follow-up examination in other institutions; and, group C, 319 patients found to have HCC incidentally or because of symptoms.

Surveillance program

Surveillance of 211 subjects in group A included patients of all ages, those with chronic hepatitis and cirrhosis, patients with a background of infection by HCV or HBV, and those suffering from alcoholism or other chronic liver diseases. The surveillance program was based on US examination and AFP determination every 3 months. The need for concomitant examination by CT, MRI and DCP was decided by the referring physician (hepatologist or gastroenterologist). During the subsequent surveillance period, imaging and tumor marker studies, together with physical examinations and routine biochemical testing, were repeated every 3 months. The 544 patients of group B showed nodular

liver lesions or elevated AFP or DCP during periodic follow up in other institutions performed at approximately 6-month intervals. The classification of 319 patients into group C was based on a nodular liver lesion detected incidentally or at examination for symptoms and on patient interview, but not at periodic follow-up examination.

Treatment strategy

When a diagnosis of HCC was established at Kurume University School of Medicine, the following treatment options were assessed:

- 1 LT was only considered after 2003 and was based on HCC meeting the Milan criteria²⁰ with Child–Pugh class C cirrhosis, as set by the health insurance system in Japan.
- 2 HR was particularly assessed in patients with localized HCC and preserved hepatic reserve capacity.
- 3 Non-surgical treatments, such as PEI, microwave coagulation therapy (MCT), RFA, transarterial chemoembolization (TACE), hepatic arterial infusion chemotherapy (HAIC), systemic chemotherapy and radiotherapy were assessed when LT and HR were contraindicated or when the patient refused surgical treatment. The most appropriate therapeutic procedure was selected according to the tumor status and underlying cirrhosis. LAT such as PEI, MCT and RFA was considered in patients with one to three tumor nodules of 30 mm or less in diameter that were devoid of vascular invasion and not associated with extrahepatic metastasis. TACE, HAIC and systemic chemotherapy or radiotherapy were considered in patients with a maximum tumor size of 30 mm, more than three tumors, presence of vascular invasion and/or presence of extrahepatic metastasis.
- 4 Best supportive care was assessed when the patient had little hepatic reserve capacity or when the patient refused any treatment for the HCC.

Outcome measures

Outcome measures were compared between the two 6-year periods. In January 1995 to December 2000 and January 2001 to December 2006, 512 patients (79 of group A, 271 of group B, 162 of group C) and 562 patients (132 of group A, 273 of group B, 157 of group C), respectively, were diagnosed with HCC. In each group, we compared the following parameters between periods: (i) hepatic function tests and Child–Pugh class; (ii) tumor characteristics including size and number of HCC nodules, presence of vascular invasion and presence of extrahepatic metastasis; (iii) Milan criteria for

HCC (single nodule ≤ 50 mm in diameter or two to three tumor nodules, each measuring ≤ 30 mm in diameter), that were devoid of vascular invasion and not associated with extrahepatic metastasis);²⁰ (iv) treatment of HCC; and (v) cumulative survival of patients with HCC.

Statistical analysis

We used the χ^2 -test,² Fisher's exact and Mann–Whitney *U*-tests, where appropriate, to evaluate differences in clinical features of patients and in tumor characteristics. Survival was analyzed by the Kaplan–Meier method and survival curves were compared by the log–rank test. Survival was confirmed up to 30 September 2007. Data were analyzed using the statistical software package SPSS for Windows ver. 10.0. *P* < 0.05 was considered significant.

RESULTS

Clinical features of patients

TABLE 1 SUMMARIZES the clinical profile of the 1074 patients with HCC. Child–Pugh class A was reported in 141 group A patients (67%), 363 of group B (67%) and 228 of group C (71%). Patients with cirrhosis numbered 174 in group A (82%), 427 in group B (78%) and 213 in group C (67%). The median tumor sizes in groups A–C were 18.0, 24.0 and 50.0 mm, respectively.

Of the 1074 patients, 650 (61%) with HCC met the Milan criteria, including 192 of group A (91%), 374 of group B (69%) and 84 of group C (28%). With regard to treatment, none of the patients received LT, while 27 (13%), 65 (12%) and 43 (13%) of group A, B and C patients, respectively, were treated with HR. Furthermore, 147 (69%), 265 (49%) and 46 (15%) patients in groups A, B and C, respectively, were treated by LAT, including PEI, MCT and RFA, while 31 (15%), 196 (36%) and 213 (67%) patients in groups A, B and C, respectively, were treated with interventional radiology (IVR) including TACE and HAIC, systemic chemotherapy or radiotherapy. Six (3%), 18 (3%) and 17 (5%) patients in groups A, B and C, respectively, were followed up conservatively without any specific treatment for HCC because of hepatic failure or patient refusal of treatment for HCC.

Comparison between 1995 and 2000 and 2001–2006

Tables 2–4 summarize the comparison of groups A–C patients between January 1995 and December 2000, and January 2001 and December 2006. For group A, male : female ratio, age, background liver disease, cirrhosis, serum levels of prothrombin activity, total bilirubin, AFP and DCP were not different between the two periods, while serum albumin levels and the frequency of Child–Pugh class A were significantly higher in

Table 1 Clinical profile of 1074 patients with hepatocellular carcinoma

	Group A	Group B	Group C
Number of patients	211	544	319
Sex (M/F)	124/87	373/171	270/49
Age (median [range])	67 (49–86)	67 (16–88)	64 (29–87)
Background (HCV/HBV/HCV[–] and HBV[–])	179/18/14	454/54/36	214/59/46
Prothrombin activity (%; median [range])	81 (35–130)	79 (24–130)	83 (30–130)
Total bilirubin (mg/dL; median [range])	1.0 (0.3–3.3)	1.0 (0.2–12.5)	1.0 (0.1–20.0)
Albumin (g/dL; median [range])	3.6 (1.8–5.1)	3.5 (1.8–4.8)	3.5 (2.1–4.6)
Child–Pugh class (A/B or C)	141/70	363/181	228/91
Cirrhosis (yes/no)	174/37	427/117	213/106
AFP (ng/mL; median [range])	17 (1–195741)	39 (1–883828)	72 (1–2397149)
DCP (<100/≥100 mAU/mL)	173/38	371/173	110/209
Tumor size (mm; median [range])	18.0 (7–99)	24.0 (8–140)	50.0 (9–300)
Tumor number (1/2–3/≥4)	137/61/13	275/166/103	81/87/151
Vascular invasion (yes/no)	4/207	37/507	87/232
Extrahepatic metastasis (yes/no)	1/210	7/537	36/283
Milan criteria (met Milan/outside Milan)	192/19	374/170	84/235
Treatment (HR or LAT/IVR or supportive care)	174/37	330/214	89/230

HCV, hepatitis C virus; HBV, hepatitis B virus; AFP, α -fetoprotein; DCP, des- γ -carboxy prothrombin; HR, hepatic resection; LAT, locoregional ablative therapies; IVR, interventional.

Table 2 Comparison of 1995–2000 and 2001–2006 data of 211 patients of group A

	1995–2000	2001–2006	<i>P</i> -value
Number of patients	79	132	
Sex (M/F)	44/35	80/52	<i>P</i> = 0.483
Age (median [range])	66 (49–80)	67 (49–86)	<i>P</i> = 0.352
Background (HCV/HBV/HCV[–] and HBV[–])	71/3/5	108/15/9	<i>P</i> = 0.156
Prothrombin activity (%; median [range])	79 (51–130)	82 (35–115)	<i>P</i> = 0.090
Total bilirubin (mg/dL; median [range])	1.1 (0.4–3.0)	0.9 (0.3–3.3)	<i>P</i> = 0.089
Albumin (g/dL; median [range])	3.4 (1.8–4.4)	3.7 (2.4–5.1)	<i>P</i> = 0.004
Child–Pugh class (A/B or C)	46/33	95/37	<i>P</i> = 0.040
Cirrhosis (yes/no)	69/10	105/27	<i>P</i> = 0.191
AFP (ng/mL; median [range])	20 (3–5765)	16 (1–195741)	<i>P</i> = 0.129
DCP (<100/≥100 mAU/mL)	61/18	112/20	<i>P</i> = 0.163
Tumor size (mm; median [range])	18.0 (7–50)	18.5 (8–99)	<i>P</i> = 0.406
Tumor number (1/2–3/≥4)	48/25/6	89/36/7	<i>P</i> = 0.581
Vascular invasion (yes/no)	0/79	4/128	<i>P</i> = 0.118
Extrahepatic metastasis (yes/no)	0/79	1/131	<i>P</i> = 0.438
Milan criteria (met Milan/outside Milan)	72/7	120/12	<i>P</i> = 0.955
Treatment (HR or LAT/IVR or supportive care)	55/24	119/13	<i>P</i> < 0.0001

HCV, hepatitis C virus; HBV, hepatitis B virus; AFP, α -fetoprotein; DCP, des- γ -carboxy prothrombin; HR, hepatic resection; LAT, locoregional ablative therapies; IVR, interventional.

patients diagnosed during the latter period than in those detected earlier. Tumor characteristics including size, number, vascular invasion, extrahepatic metastasis and Milan criteria for HCC were comparable between the two periods. However, the frequency of receiving promising treatment was significantly higher in the latter

6 years than in the former 6 years (Table 2). For group B, significantly older age, higher frequency of HCV- and HBV-unrelated HCC and Child–Pugh class A, higher serum levels of prothrombin activity, albumin and DCP, and lower serum levels of total bilirubin and AFP were noted in the latter 6 years than in the former 6 years.

Table 3 Comparison of 1995–2000 and 2001–2006 data of 544 patients of group B

	1995–2000	2001–2006	<i>P</i> value
Number of patients	271	273	
Sex (M/F)	190/81	183/90	<i>P</i> = 0.439
Age (median [range])	66 (16–87)	69 (32–88)	<i>P</i> = 0.001
Background (HCV/HBV/HCV[–] and HBV[–])	232/29/10	222/25/26	<i>P</i> = 0.022
Prothrombin activity (%; median [range])	77 (24–130)	81 (36–122)	<i>P</i> = 0.011
Total bilirubin (mg/dL; median [range])	1.0 (0.2–7.8)	0.9 (0.3–12.5)	<i>P</i> = 0.024
Albumin (g/dL; median [range])	3.5 (2.1–4.6)	3.7 (1.8–4.8)	<i>P</i> < 0.0001
Child–Pugh class (A/B or C)	169/102	194/79	<i>P</i> = 0.031
Cirrhosis (yes/no)	222/49	205/68	<i>P</i> = 0.060
AFP (ng/mL; median [range])	52 (1–124714)	31 (2–883828)	<i>P</i> = 0.001
DCP (<100/≥100 mAU/mL)	196/75	175/98	<i>P</i> = 0.040
Tumor size (mm; median [range])	22.0 (8–105)	25.0 (9–140)	<i>P</i> < 0.0001
Tumor number (1/2–3/≥4)	122/86/63	153/80/40	<i>P</i> = 0.012
Vascular invasion (yes/no)	15/256	22/251	<i>P</i> = 0.242
Extrahepatic metastasis (yes/no)	2/269	5/268	<i>P</i> = 0.258
Milan criteria (met Milan/outside Milan)	184/87	190/83	<i>P</i> = 0.669
Treatment (HR or LAT/IVR or supportive care)	140/131	190/83	<i>P</i> < 0.0001

HCV, hepatitis C virus; HBV, hepatitis B virus; AFP, α -fetoprotein; DCP, des- γ -carboxy prothrombin; HR, hepatic resection; LAT, locoregional ablative therapies; IVR, interventional.

Table 4 Comparison of 1995–2000 and 2001–2006 data of 319 patients of group C

	1995–2000	2001–2006	P value
Number of patients	162	157	
Gender (M/F)	143/19	127/30	<i>P</i> = 0.068
Age (median [range])	65 (29–83)	64 (32–87)	<i>P</i> = 0.760
Background (HCV/HBV/HCV[–] and HBV[–])	118/28/16	96/31/30	<i>P</i> = 0.037
Prothrombin activity (%; median [range])	82 (32–130)	85 (30–120)	<i>P</i> = 0.190
Total bilirubin (mg/dL; median [range])	1.0 (0.3–7.9)	0.9 (0.1–20.0)	<i>P</i> = 0.512
Albumin (g/dL; median [range])	3.5 (2.1–4.4)	3.6 (2.1–4.6)	<i>P</i> = 0.099
Child–Pugh class (A/B or C)	112/50	116/41	<i>P</i> = 0.348
Cirrhosis (yes/no)	106/56	107/50	<i>P</i> = 0.636
AFP (ng/mL; median [range])	78 (2–976554)	72 (1–2397149)	<i>P</i> = 0.877
DCP (<100/≥100 mAU/mL)	53/109	57/100	<i>P</i> = 0.500
Tumor size (mm; median [range])	50.0 (9–180)	51.0 (10–300)	<i>P</i> = 0.363
Tumor number (1/2–3/≥4)	39/48/75	42/39/76	<i>P</i> = 0.616
Vascular invasion (yes/no)	38/124	49/108	<i>P</i> = 0.120
Extrahepatic metastasis (yes/no)	18/144	18/139	<i>P</i> = 0.920
Milan criteria (met Milan/outside Milan)	43/119	41/116	<i>P</i> = 0.931
Treatment (HR or LAT/IVR or supportive care)	43/119	46/111	<i>P</i> = 0.583

HCV, hepatitis C virus; HBV, hepatitis B virus; AFP, α -fetoprotein; DCP, des- γ -carboxy prothrombin; HR, hepatic resection; LAT, locoregional ablative therapies; IVR, interventional.

Tumor characteristics were contradictory, with significantly larger size tumors, but smaller numbers of HCC detected in the latter 6 years than in the former 6 years. The frequencies of vascular invasion, extrahepatic metastasis and Milan criteria for HCC were not different between the two periods. Finally, the frequency of receiving promising treatment was significantly higher in the latter 6 years than in the former 6 years (Table 3). For group C patients, liver function tests, Child–Pugh class, tumor characteristics, Milan criteria for HCC and treatment of HCC were comparable between the two periods; the only difference was a higher frequency of HCV- and HBV-unrelated HCC in the latter 6 years than in the former 6 years (Table 4).

Comparison of LAT between 1995 and 2000 and 2001–2006

Locoregional ablative therapies were used to treat 196 and 262 patients in 1995–2000 and 2001–2006, respectively. In the former 6 years, 140 (72%; 37 of group A, 85 of group B and 18 of group C), 32 (16%; six of group A, 24 of group B and two of group C) and 24 (12%; five of group A, 12 of group B and seven of group C) patients were treated with PEI, RFA and MCT, respectively. In the latter 6 years, none of the patients were treated with MCT, while 18 (7%; 11 of group A, six of group B and one of group C) and 244 (93%; 88 of group A, 138 of group B and 18 of group C) patients were treated with

PEI and RFA, respectively. The frequency of receiving RFA was significantly higher in the latter 6 years than in the former 6 years.

Comparison of survival rates between 1995–2000 and 2001–2006

The cumulative survival rates between the two periods according to the manner of HCC detection are shown in Figures 1 and 2. For the surveillance (+) group (groups A and B, 755 patients), the 2-, 3- and 4-year cumulative survival rates were 83%, 71% and 58% for the latter 6 years (405 patients) and 75%, 61% and 50% for the former 6 years (350 patients), respectively (Fig. 1). The cumulative survival rates of those patients in whom HCC was detected in the latter 6 years tended to be better than those diagnosed during the former 6 years. For the surveillance (–) group (group C), the cumulative survival rates were not different between the two periods (*P* = 0.5546) (Fig. 2).

DISCUSSION

OUR STUDY WAS designed to evaluate the effect of recent improvement in management of HCC according to the method applied for detection of HCC (group A, surveillance at Kurume University School of Medicine; group B, surveillance at other institutions; group C, control group).

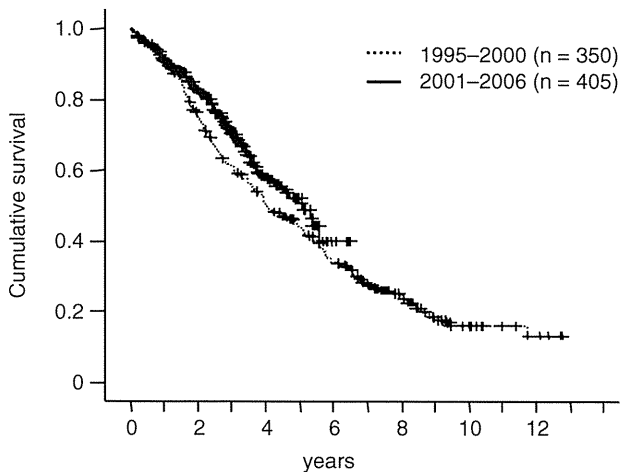


Figure 1 Kaplan–Meier survival curves of 755 patients with hepatocellular carcinoma detected by surveillance (groups A and B) in 1995–2000 and 2001–2006. The cumulative survival rate of those patients diagnosed in the latter 6 years (2001–2006) was significantly better than those diagnosed during the former 6 years (1995–2000) ($P = 0.0349$).

Sangiovanni *et al.*⁷ reported that in the last quinquennium of 1987–2001, survival of HCC patients during surveillance increased as a consequence of improved early detection of the cancers,^{4–13} wider application of radical therapies to accurately selected patients^{2,16–23} and efficient management of liver-disease complications.^{2,24,25} In the present study, improvement of surveillance did not translate into early detection of HCC in the

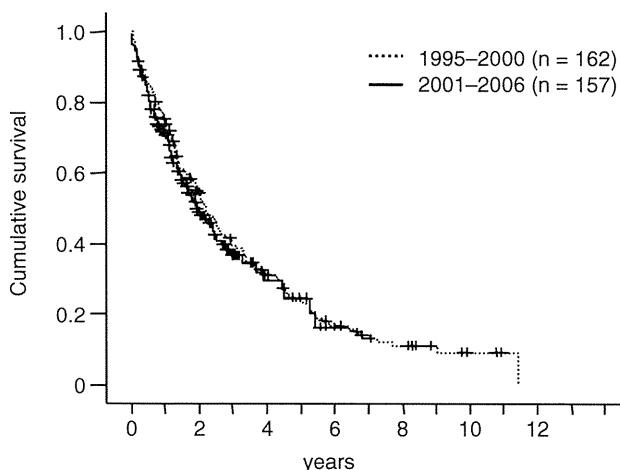


Figure 2 Kaplan–Meier survival curves of 319 patients with hepatocellular carcinoma detected incidentally or because of symptoms. The cumulative survival rates were comparable between the two periods ($P = 0.5546$).

latter 6 years (2000–2006) compared with the former 6 years (1995–2000) in either surveillance group (A or B; Tables 2,3). Surveillance for HCC based on US and AFP determination may have limited value in early detection of HCC despite the intense surveillance program in Japan. However, the frequency of patients with Child–Pugh class A and those receiving promising treatment increased more in the latter 6 years than in the former 6 years. Furthermore, the cumulative survival rates in surveillance groups in the latter 6 years tended to be better than those for patients from the former period (4-year, 58% vs 50%; Fig. 1). In Group C (control group), hepatic reserve capacity, tumor characteristics, receiving promising treatment and cumulative survival were not different between the two periods (Table 4, Fig. 2).

Based on recent technological improvements in LAT, RFA is superior to PEI with regard to the achievement of complete tumor necrosis and increase in the survival chance of patients with early-stage HCC.^{22,23} In the present study, the proportion of patients receiving RFA was significantly higher in the latter period compared to the earlier one. The change from PEI to RFA in the LAT treatment contributed to this improved survival in patients with HCC detected during surveillance. Moreover, Kurume University School of Medicine provided IFN therapy for patients with cirrhosis, in addition to follow up after curative treatment of HCC, and management of cirrhosis-related complications by nutritional therapists in the latter 6 years. Proper management of cirrhosis complications including IFN therapy for patients with cirrhosis and patients with HCC following promising treatment^{26,27} and nutrition therapy provided by nutritional therapists^{24,25} could have contributed to the increased hepatic reserve capacity and possibly survival of patients with HCC detected during surveillance.

Ultrasonography and AFP determination every 6 months for cirrhotic patients is a convenient and cost-effective surveillance program.^{7–12,14–16} In the present study, US and AFP determinations were performed every 3 months for patients with chronic liver disease (including chronic hepatitis and cirrhosis). The surveillance program in the present study may be too intensive and not as cost effective. However, the median tumor size of group A was only 18.0 mm and 192 of the 211 patients (91%) met the Milan criteria for HCC. Recent progress in available treatments for early-stage HCC and in the management of cirrhosis have made such surveillance programs more important for the early detection of HCC. Randomized prospective trials are needed to determine whether surveillance for HCC can improve survival of patients with chronic liver disease.

In conclusion, patients with HCC detected in the last 6 years through surveillance were more likely to receive promising treatment and to have better prognoses than similar patients identified in 1995–2000.

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APPENDIX

Table A1 Clinical profile of 1074 patients with hepatocellular carcinoma

	Group A	Group B	Group C	Kruskal–Wallis test
Number of patients	211	544	319	
Sex (M/F)	124/87	373/171	270/49	
		$P = 0.011$	$P < 0.0001$	
			$P < 0.0001†$	
Age (median [range])	67 (49–86)	67 (16–88)	64 (29–87)	
		$P > 0.05$	$P < 0.05$	$P < 0.0001$
			$P < 0.05†$	
Background (HCV/HBV/HCV[–] and HBV[–])	179/18/14	454/54/36	214/59/46	
		$P = 0.842$	$P < 0.0001$	
			$P < 0.0001†$	
Prothrombin activity (%; median [range])	81 (35–130)	79 (24–130)	83 (30–130)	
		$P > 0.05$	$P > 0.05$	$P = 0.005$
			$P < 0.05†$	
Total bilirubin (mg/dL; median [range])	1.0 (0.3–3.3)	1.0 (0.2–12.5)	1.0 (0.1–20.0)	
		$P > 0.05$	$P > 0.05$	$P = 0.761$
			$P > 0.05†$	
Albumin (g/dL; median [range])	3.6 (1.8–5.1)	3.5 (1.8–4.8)	3.5 (2.1–4.6)	
		$P > 0.05$	$P > 0.05$	$P = 0.953$
			$P > 0.05†$	
Child–Pugh class (A/B or C)	141/70	363/181	228/91	
		$P = 0.980$	$P = 0.255$	
			$P = 0.147†$	
Cirrhosis (yes/no)	174/37	427/117	213/106	
		$P = 0.224$	$P < 0.0001$	
			$P < 0.0001†$	
AFP (ng/mL; median [range])	17 (1–195741)	39 (1–883828)	72 (1–2397149)	
		$P < 0.05$	$P < 0.05$	$P < 0.0001$
			$P < 0.05†$	
DCP (<100/≥100 mAU/mL)	173/38	371/173	110/209	
		$P < 0.0001$	$P < 0.0001$	
			$P < 0.0001†$	
Tumor size (mm; median [range])	18.0 (7–99)	24.0 (8–140)	50.0 (9–300)	
		$P < 0.05$	$P < 0.05$	$P < 0.0001$
			$P < 0.05†$	
Tumor number (1/2–3/≥4)	137/61/13	275/166/103	81/87/151	
		$P < 0.0001$	$P < 0.0001$	
			$P < 0.0001†$	
Vascular invasion (yes/no)	4/207	37/507	87/232	
		$P = 0.008$	$P < 0.0001$	
			$P < 0.0001†$	
Extrahepatic metastasis (yes/no)	1/210	7/537	36/283	
		$P = 0.328$	$P < 0.0001$	
			$P < 0.0001†$	
Milan criteria (met Milan/outside Milan)	192/19	374/170	84/235	
		$P < 0.0001$	$P < 0.0001$	
			$P < 0.0001†$	
Treatment	174/37	330/214	89/230	
(HR or LAT/IVR or Supportive care)		$P < 0.0001$	$P < 0.0001$	
			$P < 0.0001†$	

†Group B vs group C.

HCV, hepatitis C virus; HBV, hepatitis B virus; AFP, α -fetoprotein; DCP, des- γ -carboxy prothrombin; HR, hepatic resection; LAT, locoregional ablative therapies; IVR, interventional.

Intra-arterial therapy with cisplatin suspension in lipiodol and 5-fluorouracil for hepatocellular carcinoma with portal vein tumour thrombosis

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SUMMARY

Background

Portal vein tumour thrombosis is a negative prognostic factor for hepatocellular carcinoma (HCC).

Aim

To assess the efficacy of cisplatin in lipiodol emulsion combined with 5-fluorouracil (5-FU) for patients with HCC and portal vein tumour thrombosis.

Methods

The study subjects were 51 patients with the above-specified criteria who received injection of cisplatin suspension in lipiodol emulsion followed by intra-arterial infusion of 5-FU. The primary objective was to determine tumour response to the treatment, while the secondary objectives were safety and tolerability. Independent factors for survival were also assessed.

Results

Ten patients had complete response and 34 patients had partial response (response rate, 86.3%). The median survival for all 51 patients was 33 months, while that for 10 complete response patients and 21 patients who showed disappearance of HCC following additional therapies was 39 months. The single factor that significantly influenced survival was therapeutic effect. Treatment was well tolerated and severe toxicity was infrequent, with only grade 3 toxicity (thrombocytopenia) in one patient.

Conclusions

The present study demonstrated the efficacy of hepatic arterial infusion chemotherapy using cisplatin-lipiodol emulsion and 5-FU without serious adverse effects in patients with unresectable HCC and portal vein tumour thrombosis.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is increasing worldwide and is one of the most common malignant tumours in the tropics and the Far East, including Japan.¹ It is the sixth most common cancer worldwide with 626 000 new cases in 2002.² HCC develops multifocally in chronically damaged liver. Epidemiological studies from Japan indicate that approximately 75% of HCC are caused by hepatitis C virus (HCV) infection and 10% by hepatitis B virus (HBV) infection.³

The development of sophisticated diagnostic modalities such as computed tomography (CT), magnetic resonance imaging (MRI) and abdominal ultrasonography (US), have allowed early diagnosis of HCC. Patients with small HCC are usually treated with surgical resection, liver transplantation, percutaneous ethanol injection therapy, microwave coagulation therapy, or percutaneous radiofrequency ablation. The prognosis of patients with small HCC has improved following the application of these therapeutic modalities.⁴

On the other hand, treatment of advanced HCC includes trans-hepatic arterial chemoembolization (TACE), trans-hepatic arterial infusion chemotherapy (HAIC), systemic chemotherapy, hormonal therapy and immunotherapy. However, only TACE has been confirmed to improve long-term survival.⁵ In advanced HCC, tumour cells easily invade the portal vein.⁶ Unfortunately, despite the progress in diagnostic techniques for HCC, portal vein tumour invasion is found in 12.5–39.7% of patients with HCC.^{7–9} Portal vein tumour invasion is a crucial factor in the prognosis of patients with HCC.¹⁰ Many clinical trials for advanced HCC with portal vein tumour thrombosis have been conducted. However, two systemic reviews confirmed negative outcome of these clinical trials.^{6, 11} Two recent phase III clinical trials have shown that sorafenib, an orally available multikinase inhibitor, improves the median overall survival in patients with advanced HCC.^{12, 13} Sorafenib has antivascular properties through targeting vascular endothelial growth factor (VEGF) receptor 2 and platelet-derived growth factor (PDGF) receptor and also blocks tumour cell proliferation by targeting the Raf/MEK/ERK signalling pathway.¹⁴ However, patients with HCC and portal vein tumour thrombosis usually have very short survival and grave prognosis even when treated with sorafenib.¹⁵ In Japan, such patients have been sometimes treated with HAIC with cisplatin and 5-fluorouracil (5-FU) or 5-FU and subcutaneous interferon- α injection.^{16–19}

In the present study, we investigated the efficacy and safety of the new combination therapy of cisplatin-

lipiodol suspension and 5-FU for HCC with portal vein tumour thrombosis.

PATIENTS AND METHODS

Criteria for treatment

The following criteria were used for the use of cisplatin-lipiodol suspension and 5-FU: (i) tumour thrombosis invading the portal vein (Vp2–4), (ii) absence of extrahepatic metastases, (iii) patients age >20 years, (iv) estimated life expectancy >3 months, (v) platelet count >50 000/ μ L and leucocyte count >2000/ μ L, (vi) Child-Pugh class A or class B, and (vii) performance status [Eastern Cooperative Oncology Group (ECOG)] level²⁰ of 0–2. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics review committees of Yame Republic Hospital and Kurume University, School of Medicine. Informed written consent was obtained from each patient before enrolment in the study.

Patients

From July 2004 to February 2009, 61 consecutive patients with non-resectable HCC and portal vein tumour thrombosis (Vp2–4) were referred to Yame Republic Hospital. All patients were classified as Barcelona Clinic Liver Cancer (BCLC) stage C.²¹ Each patient underwent clinical examination, US, CT, MRI, and angiography. Extrahepatic metastases were found in seven patients and three patients were Child-Pugh class C. These 10 patients were excluded from the study. We investigated the efficacy and safety of cisplatin-lipiodol plus 5-FU therapy in the remaining 51 patients (men; 43, women; 8, age 57–85 years). All patients had liver cirrhosis (Child-Pugh class A; 26, class B; 25). Thirty-nine patients were HCV antibody-positive, six were HBs antigen-positive and six were HCV antibody-negative and HBs antigen-negative. The ECOG performance status of the 51 patients was 0 or 1. These patients were free of uncontrolled ascites and hepatic encephalopathy. Leucocyte and platelet counts were >3000/ μ L and 50 000/ μ L respectively. Serum creatinine level was <1.5 mg/dL (Table 1).

Catheter placement

After local anaesthesia, a J-shaped 4-French catheter through a 4-French introducer sheath was inserted through the femoral or brachial artery by the Seldinger method. The catheter was advanced into the target artery under fluoroscopic guidance, and visceral arteriography

Table 1 | Baseline clinical characteristics

No. of patients	51
Age (years)	68.5 ± 9.2
Male/Female	43/8
Alcohol intake (+/−)	18/33
Albumin (g/dL)	3.6 ± 0.5
Total bilirubin (mg/dL)	1.2 ± 0.7
Prothrombin time (INR)	1.2 ± 0.1
White cell count (/L)	4963 + 1894
Haemoglobin (g/dL)	12.6 ± 2.2
Platelet (×10 ⁹ /L)	150 ± 86
Child-Pugh class : A/B	26/25
HBV (+)/HCV (+)/HBV (−) and HCV (−)	6/39/6
Previous treatment (+/−)	35/16
Portal vein invasion (trunk/first branch/second branch)	11/18/22
Maximum tumour size (mm), (<100 mm/≥100 mm)	88.6 ± 32.1, 31/20
AFP (ng/mL) (≤1000/>1000, ≤10 000/>10 000)	29/10/12
AFP L3 (≥10%(%))	86.3%
DCP (mAU/mL) (≤1000/>1000, ≤10 000/>10 000)	14/20/17
Macroscopic finding (nodular/infiltrative)	15/36
Tumour location (unilobular/bilobular)	18/33

was performed to detect HCC. The right gastric artery and gastro-duodenal artery were embolized using microcoils (Diamond Coli, Boston Scientific; Trufill, Cordis; or Hilal Embolization Microcoils, Cook Europe) to prevent gastroduodenal injury by anti-cancer agents. A polyurethane-covered catheter (Anthon P-U Catheter, Toray Medical, Tokyo, Japan) was used as the indwelling catheter. The tip of the catheter was placed in the common hepatic artery or proper hepatic artery. The other end of the catheter was connected to the injection port and the device was implanted in a subcutaneous pocket. To prevent obstruction of the catheter, 5 mL (5000 U) of heparin solution was injected biweekly via the injection port.

Treatment protocol

The cisplatin-lipiodol plus 5-FU regimen comprised a combination of 50 mg cisplatin in 5–10 mL lipiodol and continuous infusion of 5-FU (1500 mg/5 days). At day 1 of treatment, cisplatin with lipiodol was injected through

the reservoir catheter followed by 5-FU (250 mg). Then, 5-FU (1250 mg) was continuously infused using a balloon pump (SUREFUSER PUMP, Nipro Pharma Corporation, Osaka, Japan) for 5 days. This regimen was applied once a week during the first 2 weeks of admission, then the combination of 20 mg cisplatin with lipiodol and 5-FU (500–1250 mg) was infused every 2 weeks at the out-patient department (OPD) as long as possible. Chemotherapy was discontinued when adverse effects reached level 2 of the ECOG classification with the exception of platelet and leucocyte counts of <30 000/ μ L and 2000/ μ L respectively.

Assessment of response to chemotherapy

The primary efficacy endpoint was objective tumour response, while the secondary endpoint was patient survival. The primary efficacy endpoint was assessed at 3 months after the initial treatment and then every 2 months. At 3 months after initial treatment, partial responders and complete responders were distinguished. Tumours were bi-dimensionally measured by dynamic CT or dynamic MRI. The response to treatment was evaluated according to the Response Evaluation Criteria in Solid Tumours (RECIST)²² and following the EASL²³ amendments that take into account the amount of necrotic tumour: as complete response (CR), all measurable lesions disappeared for more than 4 weeks; partial response (PR), sum longest diameter decrease more than 30% and no new lesion for more than 4 weeks; progressive disease (PD), sum longest diameter increase more than 25% or appearance of new lesion; stable disease (SD), no definition of PR and PD for more than 8 weeks.

Assessment of tolerability

Safety was assessed at each study visit, by adverse events, a brief physical examination, vital sign measurements and clinical laboratory evaluation. The severity of any toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria, version 3. The presence of seven clinical symptoms and signs commonly noted in patients with HCC (ascites, anorexia, jaundice, local pain, lack of energy, malaise or bodily discomfort-fatigue and intratumoural haemorrhage) and complications associated with indwelling catheter (e.g. gastro-duodenal ulcer, infection, thrombosis and vascular damage) were also assessed.

Statistical analysis

Baseline data were expressed as mean ± s.d. or as median and range values. Survival was confirmed up to

31 August 2009. Cumulative survival was calculated using the Kaplan–Meier method and compared by the log rank test. Independent factors for survival were assessed with the Cox proportional hazard regression model. Statistical significance was defined as a *P* value less than 0.05. The SPSS software version 14.0J (SPSS inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

Tumour characteristics

All patients were followed-up for more than 6 months. Tumour thrombosis was noted in the main portal vein in 10 patients, in the first branch in 18, and in the 2nd branch in 23 patients. The mean diameter of the main tumour was 87.0 mm (range, 50–170 mm). Serum α -fetoprotein (AFP) levels in 41 patients were >20 ng/mL. AFP-L3 was positive (>10%) in 32 patients, and 44 patients were des- γ -carboxy prothrombin (DCP)-positive (>40 AU/mL). Patients received 2–28 (median, 8.7) courses of cisplatin-lipiodol plus 5-FU therapy (Table 1).

Response to cisplatin-lipiodol plus 5-FU therapy and additional therapy

Of the 51 patients treated with this regimen, 10 (19.6%), 34 (66.7%) and 5 (9.8%) patients had a CR, PR and SD respectively [response rate (CR + PR/51) = 86.3%]. The remaining patient had PD. Of the 34 patients with PR, 24 were treated with surgical resection, RFA or TACE and showed the disappearance of visible HCC.

Survival and causes of death

Figure 1 shows the cumulative survival rates of 51 patients. The 12-, 24- and 36-month survival rates for the 51 patients were 72.9%, 58.1% and 34.9% respectively. The median survival rate of these patients was 33 (range, 3–51) months. The median survival time of CR, PR and SD patients were 39 (range, 13–51) months, 31 (range, 6–48) months and 7 (range, 4–23) months respectively. There was a significant difference in the survival time of the three groups. Figure 2 shows the cumulative survival rates of CR and PR patients, and SD and PD patients. The 12-, 24- and 36-month survival rates of the CR and PR patients were 78.4%, 61.8% and 37.1% respectively. There was a significant difference in the survival time between CR and PR patients and SD and PD patients. Figure 3 shows the cumulative survival rates of 10 patients with CR and 14 patients with PR who later showed disappearance of viable HCC after additional therapy and the remaining 27 patients who failed to be

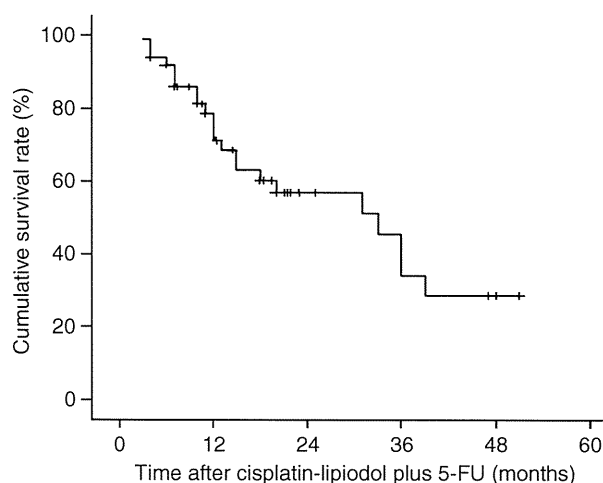


Figure 1 | Overall survival of all treated patients (*n* = 51).

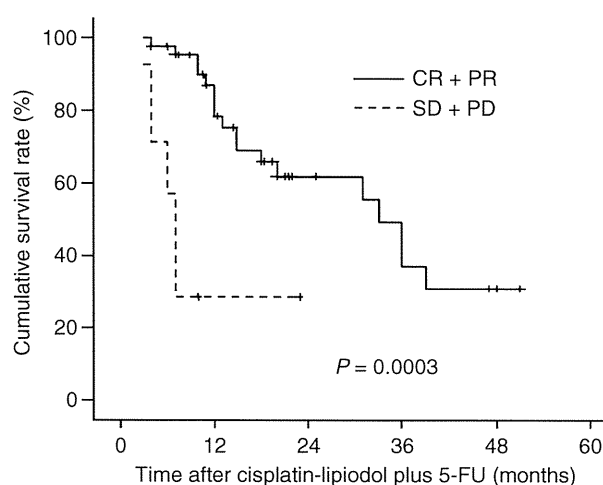


Figure 2 | Overall survival of patients who showed CR or PR and patients who showed SD or PD. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. *P* = 0.0003 by Log Rank test.

tumour-free after additional therapy. The median survival time for the 24 patients who showed disappearance of viable HCC was 39 (range, 6–51) months. The 1-, 2- and 3-year survival rates of these patients were 100%, 89.5% and 53.7% respectively. On the other hand, the median survival time and 1-, 2- and 3-year survival rates of the remaining 27 patients were 12 (range, 3–25) months, and 44.8%, 24.0% and 0% respectively. There was a significant difference in survival between patients who showed disappearance of viable HCC and those with visible HCC during the treatment. Figure 4 displays the cumulative tumour progression-free survival time. The 6-, 12- and 24-month progression-free survival

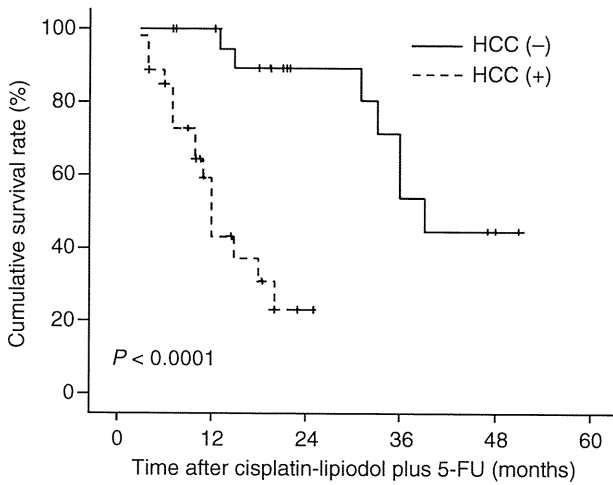


Figure 3 | Overall survival of patients who showed CR ($n = 10$) and disappearance of HCC after additional treatment ($n = 14$), and patients with variable HCC ($n = 27$). $P = 0.0001$ by Log Rank test; HCC (-), patients without variable HCC after treatment; HCC (+), patients with variable HCC after treatment; CR, complete response.

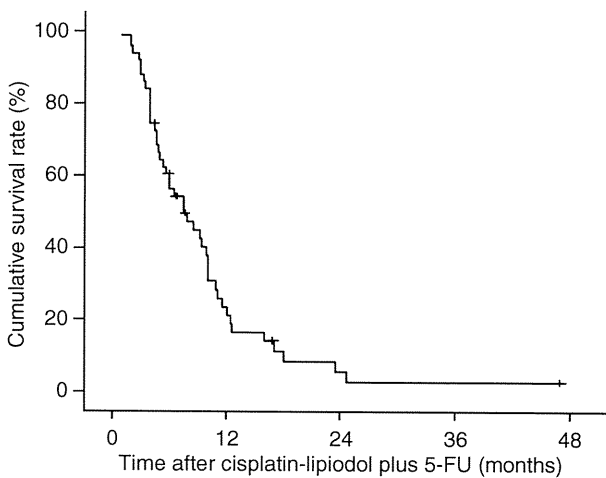


Figure 4 | Progression-free survival of all treated patients ($n = 51$).

rates of these patients were 62.1%, 21.9% and 11.7% respectively, with a median progression-free survival of 8.0 months. With regard to the relationship between survival and degree of tumour progression, there was no significant difference in median survival among patients with tumour thrombosis in the 2nd branches, the 1st branches and the portal vein trunk. With regard to the relationship between survival and liver damage, there was no significant difference in the median survival

between patients with Child-Pugh class A and those with class B. During the follow-up period, 28 patients died. Of these, 24 died of tumour progression; two of rupture of oesophageal varices, one of liver failure and one patient died of renal failure.

Two of the 13 factors analysed by univariate analysis showed prognostic significance: tumour location ($P = 0.048$) and therapeutic effect ($P < 0.001$). Multivariate analysis identified only one variable, therapeutic effect, to be an independent predictor of mortality ($P < 0.001$) (Table 2).

Regarding the therapeutic effect, two of the 13 factors analysed by univariate analysis showed the therapeutic significance: tumour location ($P = 0.042$) and grade of portal vein invasion ($P = 0.002$). Multivariate analysis identified only one variable, grade of portal vein invasion, to be an independent predictor of therapeutic effect ($P = 0.006$) (Table 3).

Adverse effects and complications

No serious complications due to indwelling catheters, such as peptic ulcer, infection, thrombus and other vascular disorders, were observed. Treatment was not dis-

Table 2 | Univariate and multivariate analyses of survival for hepatocellular carcinoma

	HR (95% CI)	P value
Univariate analysis		
Gender (male)	1.24 (0.54-2.87)	0.609
Age (>65)	1.08 (0.32-3.65)	0.899
Alcohol intake (+)	1.15 (0.49-2.66)	0.752
HCV (positive)	0.50 (0.20-1.29)	0.152
HBV (positive)	2.03 (0.56-6.23)	0.218
Child-Pugh class (B)	1.12 (0.49-2.57)	0.786
AFP(ng/mL) (>1000)	1.51 (0.67-3.43)	0.322
DCP (AU/mL)(>1000)	1.03 (0.41-2.63)	0.946
Maximum tumour size (mm) (>100)	0.82 (0.34-2.01)	0.667
Macroscopic finding (infiltrative)	0.95 (0.39-2.31)	0.901
Tumour location (bilobular)	2.56 (1.01-6.48)	0.048
Grade of portal vein invasion (trunk)	1.62 (0.60-4.39)	0.344
Therapeutic effect (CR+PR)	0.17 (0.06-0.51)	0.001
Multivariate analysis		
Therapeutic effect (CR+PR)	0.21 (0.07-0.66)	0.007

Table 3 | Univariate and multivariate analyses of therapeutic effect for hepatocellular carcinoma (CR+PR vs. SD+PD)

	Univariate analysis		
	CR+PR group (n = 44)	SD+PD group (n = 7)	P value
Gender (male/female)	38/6	6/1	0.963
Age ($\leq 65 / > 65$)	18/26	3/4	0.923
Alcohol intake (+/-)	16/28	2/5	0.689
HCV (+/-)	32/12	7/0	0.177
HBV (+/-)	6/38	0/7	0.578
Child-Pugh class (A/B)	23/21	3/4	0.703
AFP (ng/mL) ($\leq 1000 / 1000 - 10\ 000 / \geq 10\ 000$)	26/9/9	3/1/3	0.431
DCP (AU/mL) ($\leq 1000 / 1000 - 10\ 000 / \geq 10\ 000$)	14/16/14	0/4/3	0.211
Previous treatment (+/-)	14/30	1/6	0.658
Maximum tumour size (mm) ($< 100\ \text{mm} / \geq 100\ \text{mm}$)	28/16	3/4	0.411
Macroscopic finding (nodular/infiltrative)	31/13	4/3	0.664
Tumour location (unilobular/bilobular)	18/26	0/7	0.042
Grade of portal vein invasion (trunk/first branch/second branch)	6/16/22	5/2/0	0.002

	Multivariate analysis		
	Hazard ratio	(95% CI)	P value
Grade of portal vein invasion	0.105	(0.02-0.52)	0.006

continued for serious complications. However, only one patient developed grade 3 thrombocytopenia. Grade 1 appetite loss was noted in 17 patients, six patients developed grade 1 high fever and two developed grade 2 ascitic fluid accumulation. These adverse effects were controlled by medical treatment.

DISCUSSION

The prognosis of HCC patients with portal vein tumour thrombosis is very poor. Portal vein tumour thrombosis is a significant clinicopathological variable known to influence survival of patients with advanced HCC.²⁴ The median survival of untreated patients with HCC and portal vein tumour thrombosis is reported to be 2.7–4.0 months, whereas the survival of those without portal vein tumour thrombus is 24.4 months.^{25, 26} To improve this bleak outcome, various treatments have been applied. Takizawa *et al.*²⁴ reported that the longest median survival (26.0 months) was associated with surgical resection, followed by continuous HAIC (8.0 months) and transcatheter arterial infusion/TAE (5.5 months). However, the number of patients with HCC and portal vein tumour thrombosis who are suitable for surgical resection is limited. In such situation, systemic chemo-

therapy, hormonal therapy and radiotherapy have all been reported to be of limited value.²⁷ Recently, two phase 3, double-blind, placebo-controlled trials designed to assess the efficacy of sorafenib for patients with advanced HCC were conducted.^{12, 13} They demonstrated prolonged overall survival and time to progression in sorafenib-treated patients. Llovet *et al.*²¹ propose the use of sorafenib as the 1st line treatment for patients with HCC categorized as BCLC C. However, in the SHARP trial, the median overall survival time of patients treated with sorafenib was 10.7 months.¹² In another study, the median overall survival of patients with portal vein tumour thrombosis was only 5 months.¹⁵

Trans-hepatic arterial infusion chemotherapy is a reasonable drug delivery system for patients with advanced HCC because advanced HCC receives most of its blood supply from the hepatic artery and non-cancerous liver is supplied mainly by the portal vein.²⁸ HAIC seems to deliver high concentrations of chemotherapeutic agents to HCC tissues selectively with low toxicity to non-cancerous liver tissues and whole body. Several reports described the effects of HAIC with cisplatin and 5-FU or systemic interferon- α therapy with HAIC using 5-FU for HCC patients with tumour thrombosis in the 1st branches and the portal

vein trunks. Although the response rate varied from 0% to 63%, the median survival time was less than 11.8 months.^{16, 17, 19, 29–31} We also reported previously the efficacy of HAIC with cisplatin and 5-FU for HCC patients with portal vein tumour thrombosis. In that study, the response rate and median survival time were 48% and 10.2 months respectively.¹⁷ Recently, Salem *et al.*³² treated HCC patients with intra-arterial yttrium-90 microspheres. In their report, the median overall survival of patients with Child-Pugh class A and that of patients with class B who had portal vein tumour thrombosis were 10.4 and 5.6 months respectively. In the present study, the response rate was 86.3%. The degree of portal vein tumour thrombosis was a predictor of treatment effect. The overall survival rates at 12, 24 and 36 months were 72.9%, 58.1% and 34.9% respectively, and the overall median survival rate was 33 months. Univariate and multivariate analyses showed that only the therapeutic effect was an independent prognostic factor of survival. To the best of our knowledge, cisplatin-lipiodol plus 5-FU therapy is associated with the longest survival of HCC patients with portal vein tumour thrombosis in all studies reported so far. The rationale of cisplatin+5-FU regimen is that cisplatin and 5-FU have antitumour effects;³³ cisplatin has a synergistic effect as a modulator of 5-FU.³⁴ In HAIC with cisplatin and 5-FU or IFN and 5-FU, 5-FU is usually infused for 3–5 h.^{17, 19, 30} In the present study, 5-FU was continuously infused for 5 days. 5-FU does not show a dose-dependent, but time-dependent antitumour effect.³⁵ Continuous infusion of 5-FU might enhance the antitumour effect in cisplatin-lipiodol plus 5-FU therapy compared with other HAICs. Anticancer agents in lipiodol suspension are reported to exhibit a more potent antitumour effect than anticancer agent alone.³⁶ Two possible mechanisms may explain this enhanced effect; embolization of tumour artery by lipiodol, and retention and continuous release in tumour tissues of anticancer agent. In the present study, a high concentration of cisplatin (9.8–13.5 µg/g wet tissue weight) was detected in the resected tumour tissues after 132 days of cisplatin with lipiodol injection. Furthermore, the concentration of cisplatin in tumour tissues was higher than that in adjacent non-tumour tissues (data not

shown). The reason for the long median survival of patients participating in the present study could be the continuous infusion of 5-FU and use of cisplatin with lipiodol. In the present study, 24 patients showed disappearance of viable HCC (10 CR patients, 14 PR patients followed by additional therapy). The median survival time of these patients was significantly longer than that of patients with residual HCC. Cisplatin-lipiodol plus 5-FU therapy reduced tumour volume and allowed application of other treatments, thus prolonged survival even in HCC patients with PR. A recent study demonstrated that sorafenib prolongs the survival of patients with advanced HCC.¹² However, the response rate to sorafenib was extremely low.¹² Molecular targeted agents including sorafenib are probably not suitable to make tumour-free.

In the SHARP trial, approximately 52% of patients were reported to have grade 3 or grade 4 treatment-related toxicities, and the most common adverse events were diarrhoea (39%) and hand-foot skin reaction (21%).¹² As HCC is usually accompanied by liver cirrhosis, intensive chemotherapy sometimes induces severe liver damage, leucopaenia and thrombocytopenia. However, in the present study, adverse events were less frequent than those reported recently in patients who received HAIC, TACE and sorafenib treatment.^{12, 17, 37} Only one patient in our cohort developed grade 3 thrombocytopenia. Furthermore, no deterioration of liver function or evidence of liver damage was noted. Although only two patients showed a transient increase in ascites, they were well controlled by medications. Thus, the adverse effects of this treatment were not serious and controllable.

In conclusion, the present study demonstrated the efficacy of HAIC using cisplatin in lipiodol and 5-FU, and that such therapeutic regimen for unresectable HCC with portal vein tumour thrombosis is not associated with serious adverse effects. The new HAIC can potentially become the first-line treatment for unresectable HCC with portal vein tumour thrombosis, subject to confirmation through a Phase III trial by comparison with sorafenib.

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Epigallocatechin-3-gallate improves nonalcoholic steatohepatitis model mice expressing nuclear sterol regulatory element binding protein-1c in adipose tissue

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Abstract. We examined whether or not epigallocatechin-3-gallate (EGCG) improves liver injury of nonalcoholic steatohepatitis (NASH) model mice expressing nuclear sterol regulatory element-binding protein 1c (nSREBP-1c) in adipose tissue. nSREBP-1c transgenic C57BL6 mice aged 30 weeks were divided into group 1 (no treatment), group 2 (ascorbic acid alone), group 3 (ascorbic acid and 0.05% EGCG), and group 4 (ascorbic acid and 0.1% EGCG). At 42 weeks, we performed measurement of liver weight to body weight, biochemical assays, morphometry of liver specimens, immunohistochemistry for 8-hydro-2'-deoxyguanosine (8-OhdG), and Western blotting for insulin and TNF- α signalings. Ratio of liver weight to body weight in the high dose EGCG-treated group (group 4) was significantly lower than those of groups 1 and 2 ($p < 0.05$ and < 0.01 , respectively). Blood ALT, glucose, total cholesterol, and triglyceride levels of group 4 were significantly low compared with those of the EGCG-non-treated group (groups 1 and 2) ($p < 0.05$, respectively). The degrees of steatosis, inflammation, ballooning hepatocytes and Mallory-Denk bodies in group 4 significantly improved

compared with those in other groups ($p < 0.05$, respectively). The 8-OhdG immunolocalization in liver tissues of the group 4 obviously decreased compared with those of groups 2 and 3. For Western blotting, the expressions of insulin receptor substrate-1 (IRS-1) and phosphorylated IRS-1 (pIRS-1) in liver tissues of group 4 increased compared with those of groups 2 and 3. On the other hand, the expressions of pAkt, pIKK β and pNF- κ B decreased compared with those of groups 2 and 3. From these results, EGCG reduces inflammation, insulin resistance and oxidative stress, and suppresses liver injury in nSREBP-1c transgenic mice.

Introduction

Although the obesity epidemic is a worldwide phenomenon, the severity of the epidemic differs greatly from region to region. The prevalence of obesity among Japanese adults is 3.4% in males and 3.8% in females, and is around one tenth of that in the USA (1). In addition, currently in Japan almost 30% of adult males and females over 50 years old are obese. This rising incidence of obesity parallels the dramatic increase in fatty liver in these age groups. Based on annual health checks, the prevalence of fatty liver diagnosed by ultrasonography increased from 10% in 1980 to 20-40% in 2000 among adults. Thus, nonalcoholic fatty liver disease (NAFLD) is now emerging as the most common liver disease in Japan (2,3). The recent worldwide rise in the number of patients with nonalcoholic steatohepatitis (NASH) tends to parallel the increase in metabolic syndrome, which includes obesity, type 2 diabetes and hyperlipidemia (4).

NAFLD mainly comprises of simple steatosis that is considered benign, however some patients have nonalcoholic steatohepatitis (NASH), which is a clinicopathological entity characterized by the development of hepatic histological changes resembling those induced by excessive alcohol intake that occur in the absence of alcohol abuse (5). Some patients with NASH progress to end-stage liver disease, such as cirrhosis and hepatocellular carcinoma in as little as a decade, and treatment of NASH is therefore very important. However, no single agent improves the histological end points and long-term outcomes (6-8).

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Abbreviations: epigallocatechin-3-gallate, EGCG; nonalcoholic steatohepatitis, NASH; nuclear sterol regulatory element-binding protein 1c, nSREBP-1c; 8-hydro-2'-deoxyguanosine, 8-OhdG; insulin receptor substrate-1, IRS-1; phosphorylated IRS-1, pIRS-1; nonalcoholic fatty liver disease, NAFLD; nonalcoholic steatohepatitis, NASH; aspartate aminotransferase, AST; alanine aminotransferase, ALT; glycogen synthase kinase, GSK; hepatic stellate cells, HSC

Key words: nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, nuclear sterol regulatory element-binding protein 1c, green tea polyphenol

Lipodystrophic mice expressing nuclear sterol regulatory element-binding protein 1c (nSREBP-1c) in the adipose tissues show severe insulin resistance, and develop NASH. These animals have marked fatty liver accompanied by hyperlipidemia, hypoleptinemia, and hypoadiponectinemia (9). Immunoreactive 8-hydroxy-2'-deoxyguanosine was observed in the livers of these model mice, suggesting that in addition to insulin resistance, oxidative stress is involved in the development of the NASH-like lesions.

On the other hand, epigallocatechin-3-gallate (EGCG), a type of green tea polyphenol, is a major component of green tea extract, and has the effects of body-fat reduction (10) and antihyperlipidemia (11,12). Therefore, EGCG is useful for NASH patients with obesity and/or hyperlipidemia, but there are no reports indicating that EGCG shows improvement of NASH. In the present study, we examined whether or not catechins improve liver injury of NASH model mice expressing nSREBP-1c in adipose tissue.

Materials and methods

Animals and treatment. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Kurume University School of Medicine. Transgenic C57BL6 mice (Jackson Lab, ME) expressing nSREBP-1c in adipose tissue were purchased from The Jackson Laboratory (Bar Harbor, ME) (13). We identified nSREBP-1c transgenic mice by amplifying genomic DNA isolated from tails by polymerase chain reaction using a forward primer 5'-CTACATTCGCTTTCTGCAAC-3', and used heterozygous transgenic mice in the following studies. They were bred in our laboratory, mating with wild-type C57BL6 mice (Jackson Lab), in plastic cages with wood chip bedding at a temperature of 18-22°C, moisture of 40-60%, and on a 12-h light/dark cycle. They were supplied with regular mouse chow (1450 kJ/100 g, protein; 24.9 g/100 g, fat; 4.6 g/100 g, Nippon CLEA; Shizuoka, Japan) and water *ad libitum*. nSREBP-1c transgenic C57BL6 male mice aged 30 weeks, which show the typical NASH in liver histology at this age (9), were prepared and used in this study. These mice were divided into four groups [group 1, mice given distilled water alone (n=6); group 2, mice given distilled water containing 0.005% ascorbic acid, which is used to block the oxidation of EGCG (n=6); group 3, mice given distilled water containing 0.005% ascorbic acid and low dose EGCG (0.05%) (n=6); group 4, mice given distilled water containing 0.005% ascorbic acid and high dose EGCG (0.1%) (n=6)], and body weight was measured weekly for 12 weeks. After mice were anesthetized with ether at week 43, body weight was measured, blood sample was collected from inferior vena cava, and these mice were examined as follows.

Measurement of body weight and liver weight at week 43. The body and liver weights of mice in each group after sacrifice were recorded and the percentage of expression of liver weight to body weight was measured.

Biochemical assays containing serum biological markers. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, total cholesterol, triglyceride, phospholipid

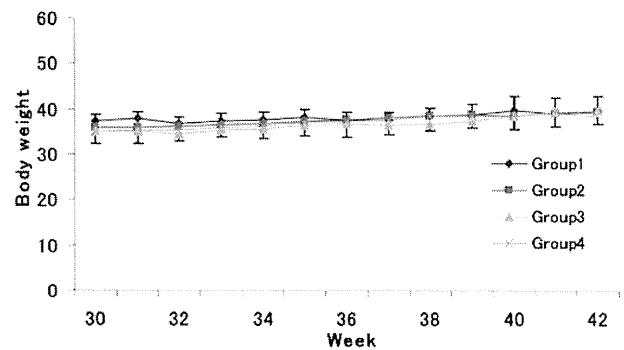


Figure 1. Changes of body weight in mice in the four groups. Body weight of mice in the four groups gradually increased throughout the course of the experiment period, but there is no significant difference among each group.

Table I. Ratio of liver weight to body weight and blood biochemical assay.

Group	1	2	3	4
Liver weight/ body weight x100 (%)	12.8±2 ^a	15.2±1.8 ^b	12.4±5.6	7.2±4.8
AST	200±132	183±8	248±153	187±57
ALT	321±260 ^a	309±47 ^a	286±257	151±67
Glucose	230±35	274±23	272±45	315±54
Triglyceride	50±23 ^a	56±19 ^a	35±25	30±7
Total cholesterol	156±26 ^a	176±17 ^a	132±54	107±42
Free fatty acid	508±171	498±68	466±116	496±126
Phospholipid	288±47 ^a	309±41 ^a	240±101	199±57

Group 1, mice given distilled water alone (n=6); group 2, mice given distilled water containing 0.005% ascorbic acid alone (n=6); group 3, mice given distilled water containing 0.005% ascorbic acid and low dose EGCG (0.05%) (n=6); group 4, mice given distilled water containing 0.005% ascorbic acid and high dose EGCG (0.1%) (n=6). EGCG, epigallocatechin-3-gallate; ^ap<0.05, ^bp<0.01 compared with group 4.

and free fatty acid levels were measured according to the manufacturer's instructions.

Histological diagnosis and morphometry of liver specimens. Paraffin-embedded sections of the liver were stained with either hematoxylin-eosin for standard microscopy and Azan-Mallory stain to observe the localization of extracellular matrix. The specimens were reviewed by two independent pathologists. Each specimen was assigned to one of the following histological subgroups for the purpose of comparative analysis, type 1, simple steatosis affecting >33% of the lobules; type 2, steatosis and lobular inflammation; type 3, steatosis and ballooning; type 4, steatosis, ballooning hepatocytes and Mallory-Denk bodies or fibrosis. We dealt with types 3 and 4 as NASH, as described previously (14). In addition, we performed morphometry of liver specimens using the histological scoring system of Kleiner *et al* (15).