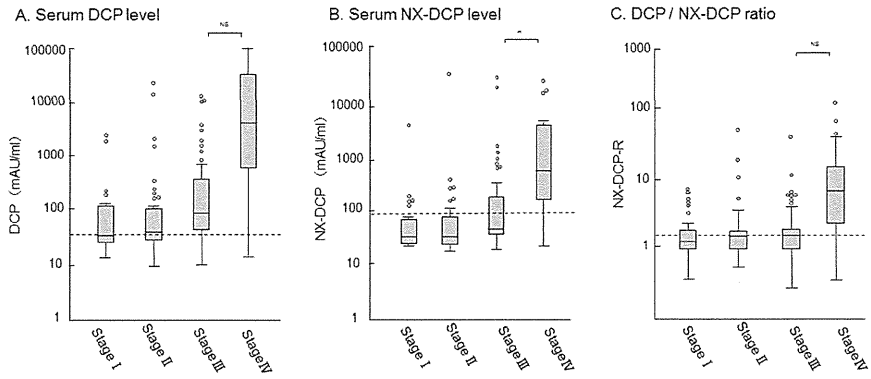

Figure 2.

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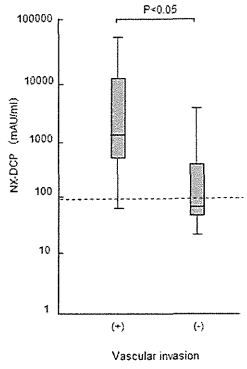
A. NX-DCP positivity and clinical parameters

	NX-DCP (+)	NX-DCP (-)	P-value
Background liver disease			
HBV	5	2	
HCV	13	5	0.99
others	6	2	
Child-Pugh classification			
A	11	6	
B	7	2	0.53
C	6	1	
Ascites			
(+)	12	4	0.65
(-)	11	6	
ALT(IU/L)			
35≤	11	5	0.71
<35	13	4	
Total bilirubin (mg/dL)			
≥2.0	17	6	>0.99
2.0<	7	3	
PT(%)			
≥70	21	7	0.60
70<	3	2	
Vascular invasion			
(+)	16	2	<0.05
(-)	8	7	

B. NX-DCP and vascular invasion



C. Overall Survival

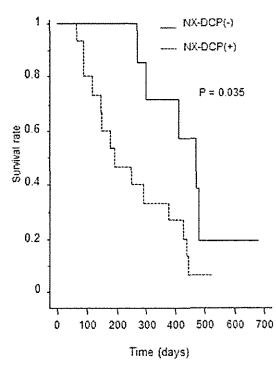


Figure 3.

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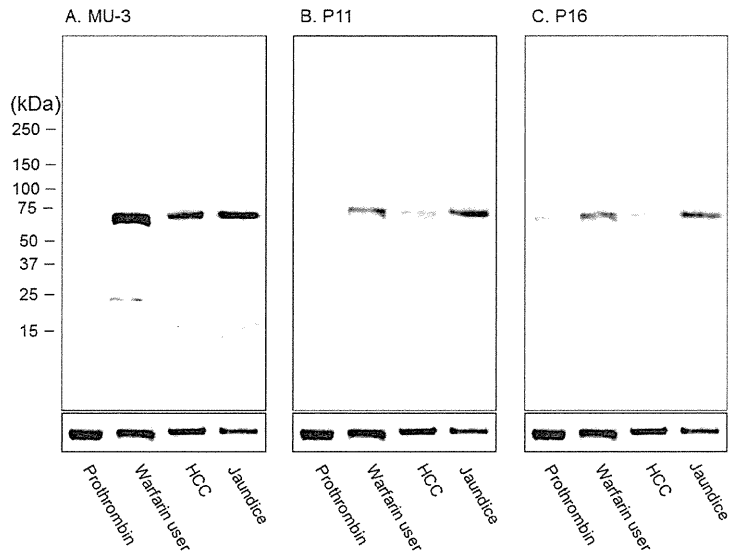

Figure 4

275x190mm (96 x 96 DPI)

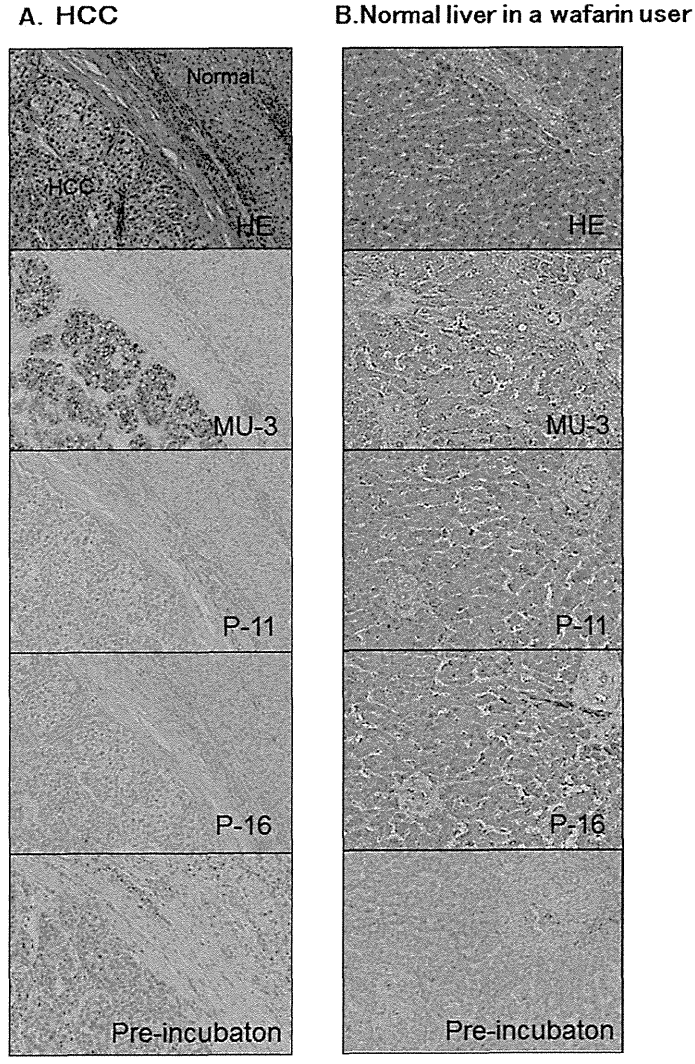

Figure 5

190x254mm (96 x 96 DPI)

Original Article

Prevention of Intrahepatic Distant Recurrence by Transcatheter Arterial Infusion Chemotherapy With Platinum Agents for Stage I/II Hepatocellular Carcinoma

Toru Ishikawa, MD; Kazuo Higuchi, MD; Tomoyuki Kubota, MD; Keiichi Seki, MD; Terasu Honma, MD; Toshiaki Yoshida, MD; and Tomoteru Kamimura, MD

BACKGROUND: The effectiveness of additional chemotherapy in preventing intrahepatic distant tumor recurrence of hepatocellular carcinoma (HCC) has not been fully established. The authors compared the efficacy of 2 platinum-based chemotherapeutic agents in combination with radical local treatment for preventing intrahepatic distant recurrence (IDR). **METHODS:** Seventy-eight patients with stage I/II HCC aged 45 to 85 years underwent transcatheter arterial chemoembolization and/or radiofrequency ablation after they received hepatic arterial infusion (HAI) of platinum compounds. The HAI consisted of cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin) in 25 patients and cis-diamminedichloroplatinum (II) (cisplatin) in 53 patients. Multivariate analysis was used to identify independent factors that were associated with IDR. **RESULTS:** Cumulative IDR rates at 1 year, 2 years, and 3 years were 21.7%, 52.2% and 75.7%, respectively, in the carboplatin group and 8.1%, 22.7%, and 36.9%, respectively, in the cisplatin group. The cisplatin group had a significantly lower IDR rate compared with the carboplatin group. The selection of a platinum agent was 1 of the independent factors for IDR in a multivariate Cox proportional hazards model. **CONCLUSIONS:** HAI chemotherapy with cisplatin before radical local treatment was effective in patients with HCC. The authors concluded that radical local treatment with concurrent HAI using cisplatin may contribute to a longer progression-free period, which could be predicted with intrahepatic imaging in patients with stage I/II HCC. *Cancer* 2011;00:000–000. © *2011 American Cancer Society.*

KEYWORDS: intrahepatic distant recurrence, transcatheter arterial infusion, cancer chemotherapy protocols, stage I/II hepatocellular carcinoma, platinum compounds.

Hepatocellular carcinoma (HCC) is associated with severe complications in patients with cirrhosis or chronic hepatitis who have severe fibrosis. Although the treatment outcome of HCC recently has improved, intrahepatic recurrence occurs at a high rate of 10% to 25% annually despite radical treatment; and, in many patients, HCC recurrence leads to fatal consequences.[1]

Previous reports have indicated that preoperative transcatheter arterial embolization (TAE) or transcatheter arterial chemoembolization (TACE) did not prevent recurrence after hepatectomy or improve patient outcomes in randomized controlled trials.[2] However, retrospective studies reported some efficacy of these techniques but with questionable significance.[3,4]

More effective chemotherapy, received preoperatively by patients with intrahepatic micrometastases or with the possibility of intraoperative tumor spread, may prevent tumor recurrence in the residual liver and further improve prognosis, although few reports have described such chemotherapy. Systemic chemotherapy generally is not effective in most patients with HCC. Chemotherapy often may impair liver function in patients who have disease complicated by cirrhosis. Currently, systemic chemotherapy is used infrequently in the treatment of HCC with cytotoxic anticancer agents. Compared with systemic chemotherapy, hepatic arterial infusion (HAI), chemotherapy has the advantages of increasing the local concentration of chemotherapeutic agents to kill cancer cells without damaging healthy liver tissue and of reducing systemic side effects.

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Cis-diamminedichloroplatinum (II) (cisplatin) is a key drug in the standard regimens for various cancers in the respiratory, digestive, and genitourinary organs.[5] Recently, treatment with cisplatin for advanced HCC has produced some encouraging results.[6-8] We previously reported on the characteristics of cisplatin treatment for HCC.[9] In Japan, a fine powder formulation of cisplatin was marketed in 2004 that allowed the preparation of a high-concentration, aqueous cisplatin solution. Many nephrotoxic and emetic effects associated with cisplatin have been reported, and cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin) has demonstrated antitumor activity comparable to that of cisplatin with fewer nephrotoxic and emetic effects than those of cisplatin. Carboplatin has been regarded as a useful anticancer agent for HCC.[10] In the current study, we investigated the efficacy of radical local treatment in combination with HAI chemotherapy with the platinum-based agents carboplatin and cisplatin in the prevention of intrahepatic distant recurrence (IDR).

MATERIALS AND METHODS

Eligibility

This was a single-center, explorative, prospective cohort study in patients with stage I/II HCC. All patients met the following criteria: unresectable disease (including rejection of hepatectomy) or difficulty tolerating surgery (age, condition of vital organs, etc), a performance status of 0 to 2, normal cardiac function on an electrocardiogram, normal renal function (normal serum creatinine, blood urea nitrogen $\leq$25 mg/dL), impaired liver function (total bilirubin 2 times the upper limit of normal, aspartate and alanine aminotransferase levels 4 times the upper limit of normal), and ages 20 to 85 years. All patients provided written informed consent. The study was approved by the local ethics committee in accordance with the 1975 Declaration of Helsinki.

Cisplatin was approved as an agent for HCC in July 2007 using phase 2 clinical trial data from studies conducted in Japan. The safety data of cisplatin were not enough at the beginning of this clinical trial. Therefore, the target number of enrolled patients was 50 in cisplatin group.

Treatment Procedure

Only 1 course of HAI with a platinum compound anticancer agent in both groups was conducted before patients underwent radical local treatment. A catheter was introduced into the proper hepatic artery by using the Seldinger technique followed by an intra-arterial infusion of either carboplatin 300 mg/m^2 over 30 minutes or of the fine powder formulation of cisplatin (IA-call; Nippon Kayaku Company Ltd., Tokyo, Japan) 65 mg/m^2 over 30 minutes. Cisplatin (100 mg per vial) was dissolved in 70 mL of normal saline that was heated to 50°C (cisplatin concentration, 1.43 mg/mL).

Next, the catheter was inserted as selectively as possible into vessels feeding the tumor, as indicated by tumor staining. Then, an epirubicin-lipiodol suspension (epirubicin [Farmorbicin]; Kyowa Hakko Kirin Company, Ltd, Tokyo, Japan; lipiodol [iodized oil]; Andre Guerget, Aulnay-sous-Bois, France) was infused according to the greatest dimension of the tumor until stasis of blood flow of the target artery was observed. In addition, we embolized the hepatic artery with a gelatin sponge to obtain complete necrosis.

A combination of a 5-hydroxytryptamine 3 antagonist with corticosteroids was administered to reduce gastrointestinal toxicity. For the prevention of renal toxicity, hydration was maintained by infusing fluid (1000 mL before the administration of cisplatin followed by 1500 mL daily for 1 week after the administration of cisplatin) to ensure sufficient diuresis.

The TACE procedure is not always an ideal radical local treatment for HCC. Kagawa et al reported that radiofrequency ablation (RFA) combined with TACE is an efficient and safe treatment that provides overall survival rates similar to those achieved with surgical resection.[11] Therefore, we believe that the additional RFA treatment contributes to complete necrosis if the patient cannot obtain a complete response with TACE. In patients who could receive an ablation therapy after TACE, RFA was given as additional ablation therapy.

Statistical Analysis

The primary endpoint of the current clinical study was IDR, which we defined as the recurrence of a new lesion developing at a different subsegment away from the previous tumor area site of radical local treatment (excluding extrahepatic metastasis). IDR rates were calculated using the Kaplan-Meier method and log-rank tests, and the generalized Wilcoxon test was used for statistical analysis. Regarding the patient characteristics, statistical analyses were performed using the Fisher exact test and the Wilcoxon rank-sum test. A Cox proportional hazards model was used to identify independent factors of IDR. The level of significance was $P < .05$. Adverse events were evaluated

Table 1. Patient Characteristics

Characteristic	Carboplatin, n=25		Cisplatin, n=53		*P*
	No. (%)	Mean±SD [Range]	No. (%)	Mean±SD [Range]	
Age, y		66.4±9.3 [46-77]		66.2±8.8 [45-84]	.724[a]
Sex					
Men	13 (52)		34 (64)		.331[b]
Women	12 (48)		19 (36)		
Stage					
I	12 (48)		25 (47)		.951[a]
II	13 (52)		28 (53)		
Multiple tumors					
Negative	20 (80)		37 (70)		.420[b]
Positive	5 (20)		16 (30)		
HCV					
Negative	7 (28)		21 (40)		.449[b]
Positive	18 (72)		32 (60)		
HBV					
Negative	22 (88)		44 (83)		.742[b]
Positive	3 (12)		9 (17)		
HBV	3 (12)		8 (15)		.784[b]
HCV	18 (72)		31 (58)		
HBV and HCV	0 (0)		1 (2)		
Others	4 (16)		13 (25)		
Child-Pugh class					
A	13 (52)		37 (70)		.302[a]
B	12 (48)		10 (19)		
C	0 (0)		6 (11)		
JIS score					
0	8 (32)		19 (36)		.700[a]
1	9 (36)		20 (38)		
2	8 (32)		12 (23)		
3	0 (0)		2 (4)		
Okuda score					
I	22 (88)		42 (79)		.343[a]
II	3 (12)		10 (19)		
III	0 (0)		1 (2)		
Platelets, $\times 10^4/\mu L$		12.1±7.2 [1.2-26.0]		12.1±5.7 [4.4-29.3]	.940[a]
Prothrombin time, %		70.3±13.8 [45.8-99.4]		74.4±15.0 [30.6-113.2]	.164[a]
AST, IU/L		63.4±32.6 [22-185]		65.4±46.0 [20-225]	.357[a]
ALT, IU/L		62.6±59.2 [12-301]		59.6±51.9 [12-264]	.915[a]
Total bilirubin, mg/dL		0.92±0.48 [0.35-2.07]		0.98±0.91 [0.28-6.45]	.818[a]
Albumin, g/dL		3.7±0.6 [2.7-5.0]		3.5±0.6 [2.2-4.4]	.353[a]
Hepatic encephalopathy					
None	21(84)		45 (85)		.925[a]
Mild	2 (8)		4 (8)		
Severe	2 (8)		4 (8)		
Ascites					
None	23 (92)		46 (87)		.475[a]
Mild	2 (8)		4 (8)		
Severe	0 (0)		3 (6)		
AFP, ng/dL		56.0±90.7 [2.3-325.2]		140.3±454.6 [1.9-2574.0]	.507[a]
PIVKA II, mAU/mL		105.5±154.2 [12-590]		88.2±261.2 [11-1700]	.012[a]

SD indicates standard deviation; HCV, hepatitis C virus; HBV, hepatitis B virus; JIS score, Japan Integrated Staging score; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II.

[a] Wilcoxon rank-sum test.

[b] Fisher exact test.

according to version 4.0 of the National Cancer Institute Common Terminology Criteria.

RESULTS

Patient Characteristics

Of 221 patients who were admitted to Saiseikai Niigata Daini Hospital for primary HCC between April 2002 and December 2008, 78 patients who were diagnosed with stage I/II HCC received HAI of platinum compounds, underwent TACE and RFA because of poor tolerance to surgery, and achieved local control. Of 78 patients (47 men and 31 women; mean age, 63 years), 25 received carboplatin between April 2002 and October 2004, and 53 received cisplatin between July 2004 and September 2008. These patients were included in the study because they could be followed over an extended period.

The patients were enrolled consecutively. We observed all patients after 1 week, when we confirmed their complete response in the local treatment area.

Although the levels of protein induced by vitamin K absence or antagonist-II (PIVKA-II) were significantly higher in the cisplatin group, there were no significant differences between the carboplatin group and the cisplatin group in patient characteristics, baseline liver function tests (including total bilirubin, albumin, and prothrombin time), clinical stage, and liver function (Table 1). The median follow-up was 36.6 months (range, 1.6-84.6 months).

Recurrence Rate

Cumulative IDR rates at 1 year, 2 years, and 3 years were 21.7%, 52.2%, and 75.7%, respectively, in the carboplatin group and 8.1%, 22.7%, and 36.9%, respectively, in the cisplatin group (log-rank test, $P = .0011$; generalized Wilcoxon test, $P = .0044$). The IDR was significantly lower in the cisplatin group (Fig. 1).

Univariate and Multivariate Analyses of Predictors of Recurrence

In univariate analysis, significant factors that affected IDR were sex, serum albumin level, prothrombin time, platelet count, and drug. In multivariate analysis, independent factors that affected IDR were drug (carboplatin vs cisplatin: hazard ratio, 0.369; 95% confidence interval, 0.174-0.781; $P = .0092$) and prothrombin time (>80% vs ≤80%: hazard ratio, 3.226; 95% confidence interval, 1.044-9.969; $P = .0418$) (Tables 2 and 3).

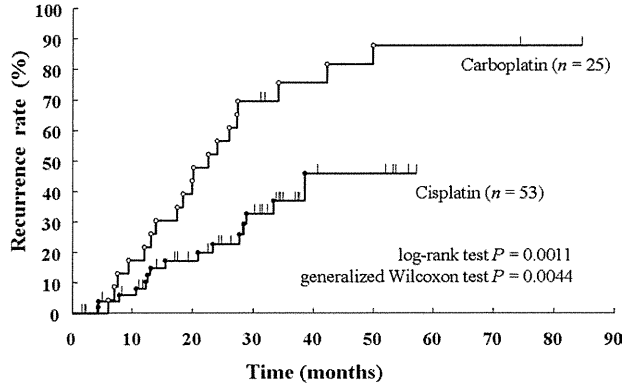

Figure 1. Cumulative intrahepatic distant recurrence rates of cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin) and cis-diamminedichloroplatinum II (cisplatin) are illustrated in patients with hepatocellular carcinoma.

Adverse Events

In the carboplatin group, grade 3 leukopenia and grade 3 thrombocytopenia were observed in 2 patients (8%) and 1 patient (4%), respectively. In the cisplatin group, 1 patient (1.8%) experienced grade 4 thrombocytopenia, and 1 patient (1.8%) experienced grade 3 thrombocytopenia. Grade 3 elevated aspartate aminotransferase levels were observed in 2 patients (3.7%), all of whom patients recovered within 1 month. There was no grade 3 or grade 4 leukopenia.

DISCUSSION

The prognosis for patients with HCC has improved dramatically with the identification of high-risk populations and the advancement of diagnostic imaging and treatment. However, recurrence of HCC is frequent in the early post-treatment period, even in patients who undergo radical hepatectomy or receive radical local treatment, including percutaneous treatment. The recurrence rate after treatment for HCC is higher than that for cancer in other organs. A possible factor is the growth of residual micrometastases in the liver that cannot be observed on imaging studies. Patients with HCC are likely to develop intrahepatic metastases through the portal vein at a relatively early stage, and there is a risk that micrometastases not observed on imaging may remain in the liver after treatment of the primary lesion. The majority of early metastases are residual liver recurrences associated with intrahepatic metastases after radical resection of HCC. Metachronous, multicentric carcinogenesis in the liver with chronic viral inflammation is a factor in tumor

Table 2. Univariate Analysis of Recurrence

Variable	Categories	*P* Log-Rank Test	Generalized Wilcoxon Test
Sex	Men/women	.0451	.0474
Albumin, g/dL	>3.5/≤3.5	.0578	.0233
Total bilirubin, mg/dL	<1.0/≥1.0	.0446	.206
Prothrombin time, %	>80/≤80	.0044	.0231
Platelets, $\times 10^4/\mu L$	≥10/<10	.0539	.0351
Hepatitis C virus	Negative/positive	.0733	.1119
Drug	Carboplatin/cisplatin	.0011	.0044

Table 3. Multivariate Analysis of Disease Recurrence: Cox Proportional Hazards Model

Variable	Categories	HR	95% CI	*P*
Sex	Men/women	1.125	0.516-2.453	.7679
Albumin, g/dL	>3.5/≤3.5	1.234	0.508-2.997	.6420
Total bilirubin, mg/dL	>1.0/≥1.0	0.940	0.338-2.617	.9058
Prothrombin time, %	>80/≤80	3.226	1.044-9.969	.0418
Platelets, $\times 10^4/\mu L$	≥10/<10	1.366	0.562-3.320	.4906
Hepatitis C virus	Negative/positive	1.505	0.608-3.727	.3770
Drug	Carboplatin/cisplatin	0.369	0.174-0.781	.0092

HR indicates hazard ratio; CI, confidence interval.

recurrence. Sakon et al[12] evaluated the recurrence rate of HCC after complete resection and reported that many recurrences within 2 years after surgery were the result of residual intrahepatic metastases; whereas, at 4 years post-surgery, recurrence was mainly the result of multicentric liver carcinogenesis. Those authors concluded that additional therapy, including HAI chemotherapy, as well as the prevention of carcinogenesis in the liver may be appropriate during the 2 years after surgery.

Several clinical trials have been performed with the aim of preventing tumor recurrence; and, in the initial reports of preoperative TACE, Imaoka and Sasaki[13] reported that, among 103 patients with HCC who underwent curative resection, the 2-year relapse-free survival (RFS) rate in 37 patients who received preoperative TACE using cisplatin was significantly better at 72% compared with an RFS rate of 46% in 14 patients who received preoperative TACE using doxorubicin, and the RFS rate was 54% in 52 patients who did not receive preoperative TACE. Zhang et al[14] reported that, in a multivariate analysis of RFS in 1457 patients who underwent hepatectomy, preoperative TACE was an independent prognostic factor and was beneficial in preventing tumor recurrence in patients who received ≥2 sessions of preoperative TACE. In a study of 100 patients who had tumors that measured <5 cm in greatest dimension, Di Carlo et al[15] observed that overall survival and RFS were significantly longer in 55 patients who received preoperative TACE than in 45 control patients. Conversely, Paye et al[16] observed no difference in overall survival or RFS between patients with identical characteristics who did and did not receive preoperative TACE. Nagasue et al[17] compared 31 patients who received preoperative TACE with 107 patients who did not receive preoperative TACE and observed complications from TACE, including severe adhesion, gall bladder infarction, liver infarction, and liver abscess in 15 patients (48%) at laparotomy. Those authors reported that preoperative TACE was not useful, because survival rates did not differ significantly between the groups. Uchida et al[18] reported that preoperative TACE increased the number of deaths from other causes, such as hepatic failure and gastrointestinal bleeding; and, in the long term, the prognosis was poor. However, a conclusion cannot easily be reached, because those reports were retrospective in nature, and the number of patients, the agents used for TACE, the number of TACE treatments, and the patient characteristics differed among groups.

We identified 2 reports on randomized clinical trials of preoperative TAE.[19,20] One was a multicenter, randomized, controlled trial conducted in Japan that examined the effects of preoperative TAE without any chemotherapeutic agents in patients with HCC who had

tumors measuring between 2 cm and 5 cm. The results from that study demonstrated that there was no significant difference in either the 5-year survival rate or the 5-year RFS rate between the preoperative TAE group and the no preoperative TAE group, and preoperative TAE produced no beneficial effects in preventing recurrence or improving prognosis after liver resection. A subgroup analysis of the necrosis rate after preoperative TAE also demonstrated no significant difference in the survival rate or the RFS rate.[20]

If the purpose of preoperative TAE (or TACE) is to prevent tumor spread arising from intrahepatic micrometastases that are present at the time of hepatectomy or from intraoperative manipulation, then it is not beneficial to perform preoperative TAE (or TACE) uniformly, regardless of whether patients have intrahepatic micrometastases. However, it may be possible to demonstrate the efficacy of preoperative TAE (or TACE) if the procedure is undergone by patients who have intrahepatic micrometastases or the possibility of intraoperative tumor spread. Controversy exists with regard to the prevention of tumor recurrence and survival rates after preoperative TAE (or TACE) for any additional treatments.

HAI chemotherapy sometimes may be chosen as a therapeutic option for HCC. TAI also is used frequently as a treatment option for advanced HCC because of poor liver function. The agents used for HAI include cisplatin, doxorubicin, epirubicin, fluorouracil, mitomycin C, mitoxantrone, and zinostatin stimalamer (a conjugate protein or copolymer of styrene-maleic acid and the antitumor protein neocarzinostatin), but there is no evidence available to define the criteria for selecting these drugs, and standard chemotherapy has not been established. Generally, HCC is less sensitive to chemotherapeutic agents, and the response rate of systemic chemotherapy is <20%. Furthermore, sufficient doses of chemotherapeutic agents cannot be given because of poor tolerability as a result of concurrent liver disease, including chronic hepatitis or cirrhosis. Therefore, standard treatment has not been established, although there are many chemotherapy regimens for HCC. We had a patient with stage IVA HCC who achieved a complete response after having enteric tegafur/uracil (UFT-E) alone, and no recurrence was observed.[21] We reported the efficacy of UFT-E in patients with stage IV HCC who received UFT-E alone, in whom interventional radiology, liver resection, TACE, chemolipiodolization, and intra-arterial infusion were not deemed feasible.[22] We also had a patient with HCC who had lung metastases who successfully responded to multimodal treatment, including cisplatin-based chemotherapy.[23,24] Thus, the selection of appropriate chemotherapeutic agents may be important in HCC. In the clinical setting, intrahepatic metastasis frequently determines prognosis even when extrahepatic metastasis is present; and, in that context, HAI is promising. Compared with systemic chemotherapy, HAI requires techniques like as catheter placement and has disadvantages, including reservoir management and a risk of damage to blood vessels caused by catheter insertion, but it allows the direct delivery of high doses of chemotherapeutic agents to the tumor site and reduces the systemic concentration of chemotherapeutic agents to a low level, which may result in a lower incidence of adverse drug reactions. Court et al[25] reported that, in cisplatin-based chemotherapy, HAI enabled greater drug accumulation within the tumor compared with systemic chemotherapy. Although there is no sufficient evidence to support the finding that HAI is more useful than systemic chemotherapy as treatment for HCC, we believe that it is necessary to deliver a high dose of chemotherapeutic agents to the liver to prevent liver recurrence from residual micrometastases, and we have used HAI as additional chemotherapy. Conventionally, anthracycline chemotherapeutic agents have been used in the HAI regimen; although, recently, platinum compounds have been used to treat HCC.[9]

Cisplatin is a chemotherapeutic agent that consists of a platinum complex compound. It is linked to DNA chains in cells through passive diffusion and active transport; then, it exerts an apoptotic effect by inhibiting DNA synthesis and subsequent cell division. The antitumor activity of cisplatin is classified into concentration-dependent, fast-acting, and slow-acting groups. The percentage of cisplatin taken up by HCC through first-pass kinetics after HAI reportedly is 48.4% (range, 34.2%-55%).[25] The local concentration of cisplatin reportedly is >10 times higher than that after intravenous administration, and cisplatin is expected to produce an enhanced therapeutic effect after selective HAI at a high dose, resulting in a concentration-dependent apoptotic effect. Unlike the chemotherapeutic agents that are excreted in bile, such as anthracyclines, cisplatin does not undergo metabolism by cytochrome P450 and is excreted mainly in urine; thus, it is considered a favorable agent that can be administered even to patients with cirrhosis who have a reduced hepatic reserve capacity.

In Japan, cisplatin was marketed in starting in 2004 as high-concentration, aqueous cisplatin solution. The reported response rate after HAI of high-concentration,

aqueous cisplatin solution was 33.8%.[26] In the future, cisplatin well may have a pivotal role in chemotherapy for HCC.[9] Cisplatin is associated with renal and auditory toxicity. In the clinical setting, carboplatin sometimes is used to reduce toxicity. Carboplatin is a cisplatin derivative that has similar antitumor activity in some regions and has been associated with less severe adverse drug reactions of gastrointestinal symptoms and renal dysfunction than cisplatin. However, carboplatin causes severe myelosuppression, especially thrombocytopenia, which is a dose-limiting toxicity of carboplatin.[5] Systemic administration of carboplatin is performed by multiple courses of intravenous infusion, each course consisting of 300 mg/m^2 to 400 mg/m^2 once daily with a 4-week washout period. Combination chemotherapy, including carboplatin, reportedly is effective in the treatment of HCC.[27-30] In the current study, we performed HAI with cisplatin and carboplatin before patients underwent radical local treatment with the aim of preventing recurrence of HCC at an early stage. We compared the 2 agents. Woo et al studied the period of IDR from the initial response to TACE treatment and observed that the 1-year, 2-year, and 3-year cumulative IDR rates patients were 27%, 45%, and 65%, respectively. In our study, the cumulative IDR rates at 1 year, 2 years, and 3 years were 21.7%, 52.2%, and 75.7%, respectively, in the carboplatin group and 8.1%, 22.7%, and 36.9%, respectively, in the cisplatin group. Our cumulative IDR rate in the carboplatin group was similar that reported by Woo et al.[31] These results suggest that HAI with carboplatin may not prevent IDR. Although both agents were administered to the whole liver, cisplatin was significantly more effective in preventing IDR in the residual liver. Although PIVKA-II status was significantly higher in the cisplatin group, considering the α-fetoprotein status, PIVKA II could be ruled out as a factor of recurrence.

In multivariate analysis, independent factors that affected IDR were drug (carboplatin vs cisplatin) and prothrombin time (>80% vs ≤80%). Prothrombin time, which reflects hepatic function, was identified as the second factor. Because patients who had positive hepatitis viral status tended to have prolonged prothrombin time (>80%, 13 of 21 patients [61.9%]; ≤80%, 48 of 57 patients [84.2%]), these patients were considered to have advanced viral cirrhosis.

Although there were more patients in our cisplatin group who received RFA treatment (n = 41, 77.4%) than in our carboplatin group (n = 13, 52%), we did not observe any statistical difference with regard to RFA status for the extent of recurrent disease ($P = .9749$). The results demonstrating a significant, preventive effect against IDR in the cisplatin group suggest the usefulness of whole-liver treatment with HAI, although a comparison with an untreated group was not made. Therefore, we conclude that radical local treatment and concurrent HAI with cisplatin may contribute to a longer progression-free period at which can be diagnosed on intrahepatic imaging studies in patients with stage I/II HCC. In the future, we expect that a prospective study of whole-liver treatment with cisplatin and an assessment of dosing frequency will contribute toward improving prognosis and preventing IDR in patients with HCC.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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ORIGINAL ARTICLE

Effect of Previous Interferon Treatment on Outcome After Curative Treatment for Hepatitis C Virus-Related Hepatocellular Carcinoma

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Abstract
Background and Aims Treatment of chronic hepatitis C virus (HCV) infection with interferon (IFN) prevents the development of hepatocellular carcinoma (HCC). The purpose of this study was to clarify the effect of previous IFN treatment before the development of HCC on recurrence and survival in HCV-related HCC patients.
Methods Three hundred ninety-five patients who underwent curative treatment for HCV-related HCC were enrolled. Of these, 124 had received IFN treatment before the development of HCC (17 achieved sustained virological response [SVR group] and 107 did not [non-SVR group]), whereas 271 patients had never received IFN treatment (IFN-untreated group). The first and second recurrence and survival rates in these patient groups were statistically analyzed.
Results The first HCC recurrence rate was similar among patient groups. In contrast, the second HCC recurrence rate was significantly lower in the SVR group than in the non-SVR group ($p = 0.003$) and the IFN-untreated group ($p = 0.006$). In multivariate analysis, platelet count ($p = 0.033$) and number of tumors ($p = 0.001$) were associated with the first HCC recurrence, while SVR ($p = 0.002$) was the only factor associated with the second HCC recurrence. The survival rate was higher in the SVR group than in non-SVR and IFN-untreated groups, and SVR to previous IFN treatment was an independent factor associated with better survival ($p < 0.001$).
Conclusions SVR to previous IFN treatment before the development of HCV-related HCC was associated with lower risk of the second recurrence of HCC and better survival.

Keywords Hepatocellular carcinoma · Hepatitis C virus · Previous interferon therapy · Recurrence · Survival

Introduction

Chronic hepatitis and cirrhosis following hepatitis C virus (HCV) infection are major risk factors for hepatocellular carcinoma (HCC) [1–3]. Particular risk factors for developing HCV-related HCC in patients are advanced stage fibrosis, male gender, older age, heavy drinking, and high serum alanine aminotransferase (ALT) levels [4, 5]. Interferon (IFN) therapy improves hepatic inflammation and inhibits the progression of hepatic fibrosis [6]. Furthermore, treating patients with IFN with chronic HCV infection can prevent the development of HCC, particularly in patients with sustained virological response (SVR) to IFN therapy [7–13]. In contrast, HCC is liable to frequently recur even after curative therapy primarily because of its multicentric occurrence, leading to a poor prognosis [14–19]. The recurrence rate after resection of HCV-related HCC is higher in patients with HCV viremia than in those without it [20]. It has been reported that IFN therapy after resection or ablation of HCC reduces recurrence and improves prognosis in patients with HCV-related HCC [21–28]. However, no complete investigation has been performed of the possible effect of IFN therapy before HCC development on the outcome of curative treatment for

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HCV-related HCC particularly in relation to the response to IFN treatment. Only a few relevant studies involving limited number of patients with previous IFN therapy are available [29–32].

The purpose of this study was to clarify the effect of previous IFN treatment before the development of HCV-related HCC on recurrence and prognosis after curative treatment of HCC in a large cohort of patients.

Patients and Methods

Patients

Between 1995 and 2006, 733 consecutive patients with HCC positive for HCV antibody and HCV RNA were diagnosed at Okayama University Hospital. Three hundred thirty-eight patients who did not receive curative treatment for HCC or undergo IFN therapy after the development of HCC were excluded from the study (Fig. 1). Inclusion criteria were as follows: (1) no evidence of HCC before consulting the Okayama University Hospital, (2) absence of hepatitis B surface antigen, (3) absence of co-existing liver diseases such as autoimmune hepatitis or primary biliary cirrhosis, and (4) absence of a history of alcohol abuse.

HCV infection was diagnosed on the basis of identification of anti-HCV antibodies using the first, second, or third enzyme-linked immunosorbent assays (Ortho Diagnostics, Tokyo, Japan). HCV RNA was identified by reverse transcription-polymerase chain reaction (RT-PCR) [33].

HCC was suspected on the basis of several imaging methods, including abdominal ultrasonography (US), dynamic computed tomography (CT), magnetic resonance imaging, and angiography. Diagnosis of HCC was confirmed by needle biopsy, by surgically resected tumor specimens, or by typical radiological findings on hepatic angiography or dynamic CT.

The study was conducted in accordance with the Helsinki Declaration and approved by the Ethical Committee of the institution.

Treatment

Of the 395 patients receiving curative treatment of HCC, 103 were treated with surgical resection and 292 with percutaneous tumor ablation (PTA) [34–37], that is, percutaneous ethanol injection therapy (PEIT) ($n = 116$), percutaneous microwave coagulation therapy (PMCT) ($n = 11$), or radiofrequency ablation (RFA) ($n = 165$). There were no patients who underwent liver transplantation or other modes of HCC treatment. The choice between surgical resection and PTA were determined according to the extent of tumor and hepatic functional reserve as assessed by Child's classification [38]. If the liver tumor consisted of fewer than three nodules that were less than 3 cm in diameter, patients were indicated

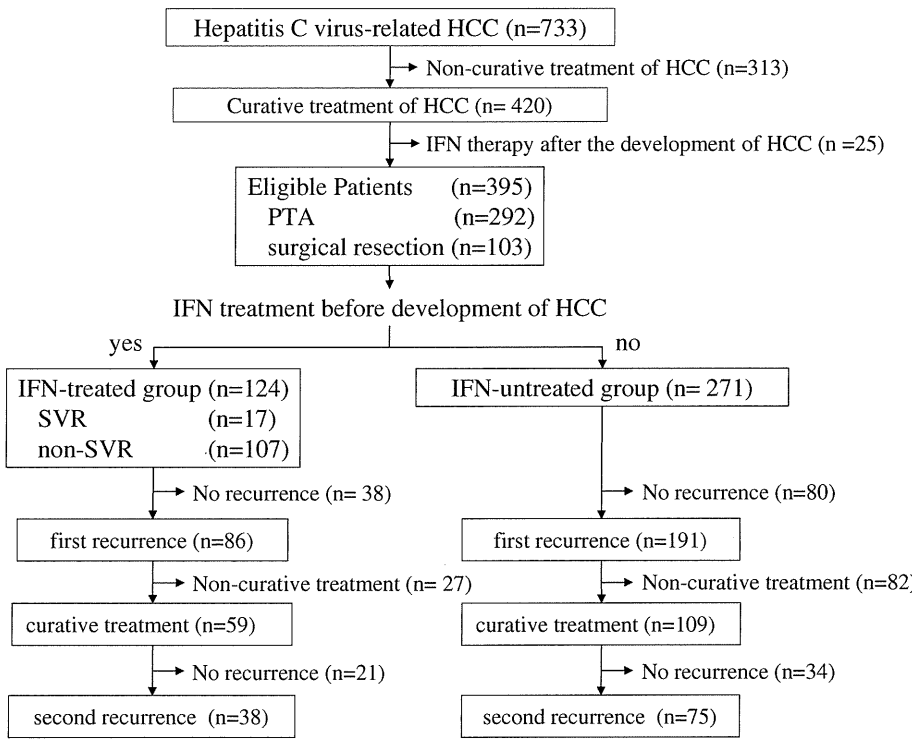

Fig. 1 Schematic presentation of patients with HCV-related hepatocellular carcinoma (HCC). Patients with HCV-related HCC who were diagnosed at Okayama University Hospital were classified into three groups according to their previous IFN treatment and response to that treatment. One hundred twenty-four patients had received IFN treatment before the development of HCC (IFN-treated group) and the remaining 271 had not (IFN-untreated group). Patients who had undergone IFN treatment before the development of HCC were further classified according to their response into a sustained virological response (SVR) group or a non-SVR group. Patients were regularly screened for HCC

for PTA. When a patient was indicated for both surgery and PTA, the modality of treatment was determined by patient choice after obtaining fully informed consent. PEIT was carried out under US guidance using a 15- or 20-cm-long needle (21 gauge) (Hakko, Chikuma, Japan) [35], PMCT was performed under US guidance using a 15-cm-long guide needle (14 gauge) according to the procedure described previously [37], and RFA was executed under US guidance using a 15- or 20-cm-long guide needle (16 gauge) (Tyco Healthcare Japan, Tokyo, Japan) [36]. PTA was repeated until complete necrosis of all HCC lesions was confirmed by dynamic CT. Treatment of HCC was considered curative, when no viable HCC lesions were detected on dynamic CT 3 months after completion of the treatment.

Of the 395 patients receiving curative treatment for HCC, 124 had received either human lymphoblastoid IFN, recombinant IFN-alpha 2a, or recombinant IFN-alpha 2b monotherapy for chronic HCV infection before the development of HCC (IFN-treated group), whereas 271 had not (IFN-untreated group) (Fig. 1). Patients received 6 million units of IFN by intramuscular injection three times weekly for 24 weeks as outpatients. If patients could not tolerate this dose, the IFN dose was reduced to 3 million units. SVR was defined as HCV RNA (as determined by RT-PCR; detection limit, 10^2 copies/ml) negativity for over 6 months after the termination of IFN therapy. SVR was achieved in 17 of the 124 patients (SVR group) and the remaining 107 were regarded as non-SVR (non-SVR group) (Fig. 1).

Follow-up of Patients

Patients attended a monthly medical consultation at the Okayama University Hospital outpatient clinic. Blood biochemical markers, including α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), were measured every 1–2 months; US was performed every 2–3 months, and dynamic CT was performed every 6 months. If HCC recurrence was suspected, further imaging examinations including dynamic CT, magnetic resonance imaging, abdominal angiography, or US-guided tumor biopsy were performed to confirm the diagnosis.

New HCC foci as well as local recurrent nodules at tumor, node, metastasis (TNM) stage I, II, and III, were mainly treated by a second course of PTA; local recurrent nodules at TNM stage IV were treated with transarterial chemoembolization or chemotherapy. Further development of HCC and survival of patients (tumor recurrence rate and survival rate) were analyzed in relation to the time interval after treatment of HCC.

Table 1 Demographic and clinical characteristics of patients with HCV-related HCC

Groups	IFN-treated		IFN-untreated (n = 271)	p^a	p^b
	SVR (n = 17)	Non-SVR (n = 107)			
Characteristics					
Sex (men/women), n	13/4	60/47	187/84	0.049	0.112
Age (years)	63 (52–71)	65 (46–82)	67 (33–85)	0.018	0.061
Laboratory data					
Total bilirubin (mg/dl)	0.74 (0.40–1.29)	0.85 (0.36–3.28)	0.91 (0.16–4.13)	0.194	0.171
Albumin (g/dl)	4.4 (3.7–4.8)	3.7 (2.5–4.8)	3.6 (2.2–4.7)	<0.001	<0.001
Prothrombin time (%)	93 (70–121)	85 (47–142)	85 (40–145)	0.355	0.023
ALT (IU/l)	22 (10–54)	55 (12–198)	60 (14–201)	0.058	<0.001
Platelet count ($\times 10^4/\mu l$)	16.6 (8.4–30.3)	9.2 (2.8–37.2)	10.1 (3.2–31.9)	0.980	<0.001
Child–Pugh stage (A/B/C), n	17/0/0	87/20/0	213/54/4	0.236	0.049
Tumor-related variables					
Number of tumors (single/multiple), n	15/2	76/31	192/79	0.603	0.136
Size of largest tumor (mm)	20 (8–40)	18 (10–53)	20 (9–74)	0.033	0.942
AFP (ng/ml)	13 (1.9–25,716)	24 (1.7–3,480)	20 (0.6–54,535)	0.956	0.297
DCP (mAU/ml)	34 (1–35,000)	46 (10–56,000)	46 (1–66,700)	0.294	0.195
Initial treatment of HCC					
PTA/surgical resection, n	6/11	79/28	207/64	0.100	0.002

Laboratory data and tumor-related variables are at the development of HCC. Continuous variables are given as medians with ranges

HCV hepatitis C virus, *HCC* hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation

[a] IFN-treated versus IFN-untreated

[b] SVR versus non-SVR

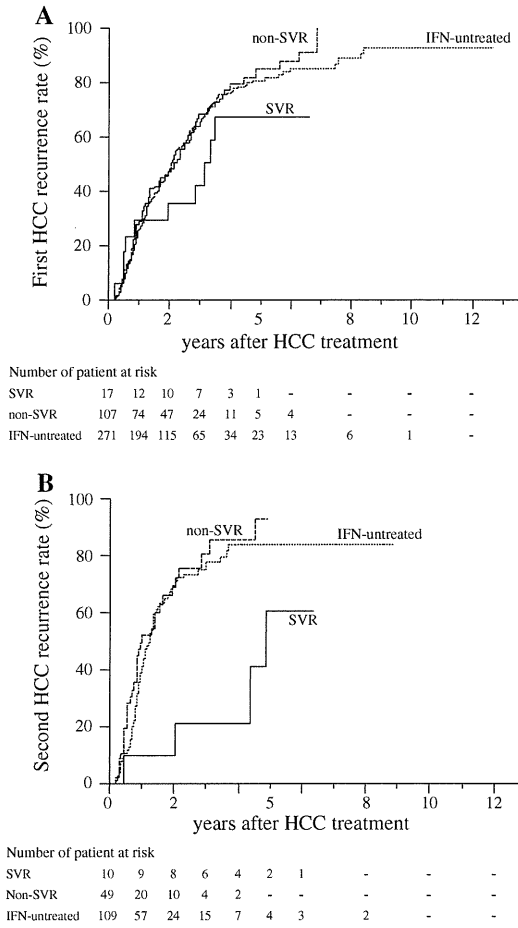

Fig. 2 Cumulative first (**a**, $n = 395$) and second (**b**, $n = 168$) HCC recurrence rates in patients with curative treatment of HCC according to the previous IFN treatment and response to the treatment. The first HCC recurrence rates were similar among SVR, non-SVR, and IFN-untreated groups (**a**). However, the second HCC recurrence rate in the SVR group at 2 years after HCC treatment was significantly lower than that in the non-SVR group (10 vs. 69%, $p = 0.003$) and the IFN-untreated group (10 vs. 70%, $p = 0.006$) (**b**)

Statistical Analysis

Statistical analysis was performed using JMP statistical discovery software, version 8.0 (SAS Institute Inc., Cary, NC). Differences between two groups were evaluated using the unpaired Student's t test or the Mann–Whitney U test. The Chi-square test or the Fisher's exact probability test was used to compare categorical data. Cumulative incidence curves were determined with the Kaplan–Meier method, and the differences between patient groups were assessed using the logrank test. Possible risk factors for recurrence of HCC and survival included both IFN-related variables and variables at the development and recurrence of HCC (age, total bilirubin level, albumin level, prothrombin time, ALT level, platelet count, number of tumors, largest tumor size, AFP level, and DCP level). Tumor associated variables, number of tumors and size of largest tumor, were transformed into categorical data consisting of two ordinal numbers by the median value. Variables exhibiting p values less than 0.10 in univariate analysis were subjected to a stepwise Cox proportional hazards regression analysis. A risk ratio with a 95% confidence interval was denoted for each analysis. p values less than 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of patients at the development of HCC are shown in Table 1. The patient group comprised 260 men and 135 women (73 men and 51 women in the IFN-treated group), and median age was 58 years (65 years in the IFN-treated group). Of the 395 patients (80%), 317 were classified as Child–Pugh stage A. Significant differences were observed between IFN-treated and untreated patients in sex, age, albumin level, and size of largest tumor. On the other hand, significant differences were observed between IFN-treated patients with SVR and non-SVR in albumin level, prothrombin time, ALT level, platelet count, Child–Pugh stage, and initial treatment of HCC. This indicated better hepatic functional reserve in SVR patients than in non-SVR patients.

The median follow-up period after curative treatment of HCC for patients with and without IFN treatment was 3.8 years and 3.5 years, respectively. In the IFN-treated group, patients underwent IFN therapy 7.2 (0.8–17.4) (median and range) years before development of HCC. Of the 395 patients, 277 (70%) had recurrence of HCC during a median follow-up period of 2.1 (1.8–2.4) years [including 86 of 124 IFN-treated patients (69%)]. Of the 168 patients receiving curative treatment for the first recurrence of HCC, 113 (67%) had a second HCC recurrence during a median follow-up period of 1.3 (1.0–1.4) years [including 38 of 59 IFN-treated patients (64%)] (Fig. 1).

HCC Recurrence Rates

The rates of the first and second HCC recurrence after curative treatment of primary HCC in each treatment group are shown in Fig. 2. In the IFN-treated group, 86 patients (10 with SVR and 76 with non-SVR) had the first HCC recurrence and 38 (four with SVR and 34 with non-SVR) had the second HCC recurrence during the follow-up period. The average times to the first and second HCC recurrence were 632 and 1,069 days, 661 and 401 days, and 666 and 428 days in SVR, non-SVR, and IFN-untreated groups, respectively. The rates of the first recurrence at 2 years in SVR, non-SVR, and IFN-untreated groups were 36, 47, and 48%, respectively. The differences between these rates were not statistically significant

($p = 0.410$) (Fig. 2a). However, the rates of the second HCC recurrence at 2 years were significantly lower in the SVR group than in the non-SVR group (10 vs. 69%, $p = 0.003$) and in the IFN-untreated group (10 vs. 70%, $p = 0.006$) (Fig. 2b). There was no significant difference in the second HCC recurrence rates between non-SVR and IFN-untreated groups ($p = 0.441$). In multivariate analysis, platelet count ($p = 0.033$) and number of tumors ($p = 0.001$) were independent factors associated with the first recurrence of HCC (Table 2), whereas SVR to previous IFN therapy ($p = 0.002$) was the only factor associated with lower risk for the second recurrence of HCC (Table 3).

Overall Survival

Survival rates after curative treatment of primary HCC in each group are shown in Fig. 3. A tendency was observed toward a higher survival rate in the IFN-treated group than in the IFN-untreated group but it was not significant ($p = 0.053$) (Fig. 3a). In contrast, survival rates at 5 years were higher in the SVR group (100%) than in non-SVR (73%) and IFN-untreated groups (62%) ($p = 0.004$) (Fig. 3b). No significant difference was observed in the survival rates between non-SVR and IFN-untreated groups ($p = 0.450$). In multivariate analysis, SVR to previous IFN therapy ($p < 0.001$), albumin level ($p = 0.006$), number of tumors ($p = 0.007$), and AFP level ($p = 0.046$) were independent factors associated with overall death after curative treatment of primary HCC (Table 4).

Discussion

In the present study, we have demonstrated that patients with SVR to previous IFN treatment before development of HCC showed lower risk for the second recurrence of HCC and better survival compared to patients with non-SVR to previous IFN treatment or IFN-untreated patients. Several studies have demonstrated that IFN therapy reduces the risk of HCC development among chronic hepatitis C patients. On the other hand, a few reports are available on the influence of previous IFN therapy before the development of HCC on patient outcomes after curative treatment of HCV-related HCC. It was initially reported that HCV-related HCC patients who received IFN therapy before development of HCC showed lower recurrence rates and better survival rates, independent of response to IFN therapy, compared to those without previous IFN therapy [29, 30]. It has recently been reported that patients showing

Table 2 Risk factors for the first recurrence of HCC ($n = 395$)

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Sex (male)	1.17 (0.91–1.51)	0.229	–	
IFN-related variables				
IFN-untreated	1			
Non-SVR	1.07 (0.82–1.39)	0.623	–	
SVR	0.68 (0.34–1.22)	0.209	–	
Variables at the development of HCC				
Age (≥60 years)	1.13 (0.84–1.56)	0.434	–	
Total bilirubin (≥1.0 mg/dl)	1.07 (0.83–1.37)	0.579	–	
Albumin (<3.5 g/dl)	1.34 (1.04–1.71)	0.022	1.24 (0.95–1.61)	0.108
Prothrombin time (<70%)	1.07 (0.79–1.43)	0.664	–	
ALT (≥40 IU/l)	1.09 (0.83–1.43)	0.542	–	
Platelet count(<10 × 10^4/μl)	1.37 (1.08–1.75)	0.009	1.34 (1.04–1.75)	0.026
Tumor-related variables				
Number of tumors (multiple vs. single)	1.66 (1.27–2.15)	<0.001	1.63 (1.24–2.14)	0.001
Size of largest tumor (≥20 mm)	1.24 (0.98–1.57)	0.074	1.22 (0.94–1.59)	0.140
AFP (≥100 ng/ml)	1.45 (1.07–1.92)	0.016	1.30 (0.96–1.74)	0.093
DCP (≥40 mAU/ml)	1.33 (1.02–1.75)	0.034	1.11 (0.85–1.44)	0.448
Initial treatment of HCC				
PTA/surgical resection	1.09 (0.84–1.43)	0.530	–	

HCC hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

Table 3 Risk factors for the second recurrence of HCC ($n = 168$)

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*
Sex (male)	1.07 (0.73–1.61)	0.719	–	
IFN-related variables				
IFN-untreated	1		1	
Non-SVR	1.17 (0.77–1.74)	0.447	1.09 (0.68–1.72)	0.718
SVR	0.27 (0.08–0.65)	0.002	0.10 (0.01–0.50)	0.002
Variables at the development of HCC				
Age (≥60 years)	1.50 (0.91–2.61)	0.115	–	
Total bilirubin (≥1.0 mg/dl)	1.08 (0.72–1.60)	0.701	–	
Albumin (<3.5 g/dl)	1.04 (0.68–1.57)	0.847	–	
Prothrombin time (<70%)	1.18 (0.70–1.89)	0.529	–	
ALT (≥40 IU/l)	1.30 (0.86–2.01)	0.220	–	
Platelet count (<10 × 10^4/μl)	1.00 (0.69–1.47)	0.984	–	
Number of tumors (multiple vs. single)	1.57 (1.04–2.32)	0.033	1.51 (0.93–2.42)	0.098
Size of largest tumor (≥20 mm)	0.91 (0.63–1.32)	0.613	–	
AFP (≥100 ng/ml)	0.65 (0.38–1.06)	0.084	0.77 (0.39–1.39)	0.391
DCP (≥40 mAU/ml)	0.81 (0.54–1.23)	0.331	–	
Initial treatment of HCC				
PTA/surgical resection	1.12 (0.75–1.69)	0.595	–	
Variables at the first recurrence of HCC				
Age (≥60 years)	0.97 (0.46–2.39)	0.950		
Total bilirubin (≥1.0 mg/dl)	0.94 (0.59–1.46)	0.785	–	
Albumin (<3.5 g/dl)	1.67 (1.06–2.61)	0.029	1.47 (0.90–2.36)	0.125
Prothrombin time (<70%)	1.24 (0.60–2.30)	0.531	–	
ALT (≥40 IU/l)	1.49 (0.95–2.40)	0.083	1.21 (0.75–2.01)	0.452
Platelet count (<10 × 10^4/μl)	1.13 (0.74–1.73)	0.573	–	
Number of tumors (multiple vs. single)	2.09 (1.37–3.13)	<0.001	1.47 (0.91–2.34)	0.112
Size of largest tumor (≥20 mm)	0.96 (0.62–1.45)	0.840	–	
AFP (≥100 ng/ml)	0.72 (0.32–1.41)	0.355	–	
DCP (≥40 mAU/ml)	1.05 (0.67–1.63)	0.842	–	

HCC hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

biochemical response, with or without SVR to previous IFN therapy, showed higher tumor-free survival rates after surgery than those without such a response to IFN or those without previous IFN therapy [31, 32]. In these previous reports, a biochemical response as well as SVR to previous IFN therapy was associated with favorable outcome, demonstrating the importance of response to previous IFN therapy for the outcome after surgery of HCV-related HCC.

However, in the present study, patients with non-SVR showed similar recurrence and survival rates as IFN-untreated patients. Furthermore, no difference was observed in the recurrence and survival rates among non-SVR patients with and without biochemical response to previous IFN therapy (data not shown). In fact, only patients with SVR to previous IFN therapy showed better outcome than those with non-SVR or IFN-untreated patients. Therefore, the present data indicate that SVR but not biochemical response without SVR to previous IFN treatment is a predictor of favorable outcome in patients who have developed HCC.

The reason for the difference between the present and previous studies in the outcome of non-SVR patients with biochemical response to previous IFN therapy is currently unknown. In patients with HCV-related chronic hepatitis and cirrhosis, who received IFN therapy and showed normalization of ALT levels, suppression of primary HCC development and better survival rates have been independently demonstrated of eradication of HCV infection by the IFN therapy [10, 11, 13, 39]. However, this

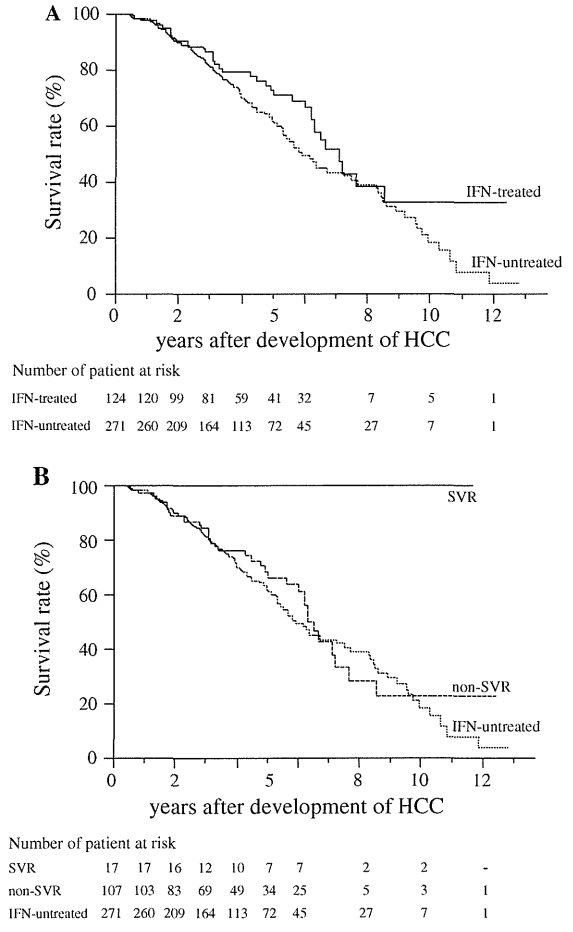

Fig. 3 Overall survival rates of HCV-related HCC patients ($n = 395$) according to their previous IFN treatment before development of HCC (**a**) and their response to the treatment (**b**). A tendency was observed toward a higher survival rate in the IFN-treated group than in the IFN-untreated group but it was not significant ($p = 0.053$) (**a**). On the other hand, the survival rate of the SVR group was significantly higher than those of non-SVR and IFN-untreated groups ($p = 0.004$) (**b**)

suppression observed for primary carcinogenesis in non-SVR patients with biochemical response to IFN therapy does not appear to be the case for secondary carcinogenesis in the present study. The period after IFN therapy was much longer in the present study than in the previous reports on primary carcinogenesis. The IFN therapy had preceded to the HCC development, that should have required long incubation after the termination of IFN treatment, and in the present study the observation of HCC recurrence and survival started with the curative treatment of the primary HCC. In patients who had sustained biochemical response but had not eradicated HCV infection, we and others demonstrated that platelet count transiently increases following IFN therapy but decrease over the following 3 years after the termination of IFN therapy. On the other hand, in patients with SVR an increase followed by persistence in platelet counts was observed [40, 41]. These observations suggest the progression of fibrosis during a longer incubation period after IFN therapy, even in the non-SVR patients with biochemical response to the therapy. Therefore, the suppressive effect of IFN therapy on development of HCC may not persist beyond the development of primary HCC particularly in these patients.

It has also been demonstrated that HCV core transgenic mice can develop HCC without apparent hepatitis [42]. Therefore, besides active hepatitis, which involves persistent hepatocyte death and regeneration, and should result in both genetic and epigenetic disorders as well as increased oxidative stress, the presence and persistence of HCV infection and viral products such as core protein may themselves play an important role in the development of HCC in non-SVR patients with biochemical response. Thus, patients with SVR who had eradicated HCV infection should have a lower incidence of HCC recurrence and higher survival rates than non-SVR patients with biochemical response.

In the present study, patients with SVR showed a better overall survival rate than other groups. However, although patients with SVR showed lower rates of the second HCC recurrence, this was not the case for the first HCC recurrence. Although both SVR and non-SVR groups have a carcinogenic background during the development of primary HCC, the carcinogenic potential in SVR patients may be gradually attenuated because of the eradication of HCV infection, whereas it may increase in those with non-SVR because of persistence of HCV infection and relapse of hepatitis, finally leading to progression of fibrosis over a longer period. However, a substantial time may be required before differences between patients with and without SVR become apparent, and these differences eventually become significant in the second recurrence of HCC.

It should also be noted that IFN-treated patients enrolled in the present and previous studies are a selected cohort, since the incidence rates of HCC development in patients treated with IFN should be lower than in those untreated with IFN [13]. This is particularly the case for patients with SVR to previous IFN treatment, whose risk for development of HCC is less than one fifth of that for IFN-untreated patients [13]. Reported risk factors for HCC development in patients who received IFN therapy include advanced fibrosis, lower platelet count, advanced age, male gender, and regular drinking [8, 9, 12, 13, 43]. Therefore, in the present study, HCC patients who received IFN therapy before the development of HCC may have demonstrated many of these characteristics, making them more prone to develop HCC than those not developing HCC after IFN therapy and not included in this study. Furthermore, it has been suggested that cirrhotic patients who develop primary

Table 4 Analysis of factors associated with overall death after curative treatment for primary HCC ($n = 395$)

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Sex (male)	1.02 (0.73–1.44)	0.911	–	
IFN-related variables				
IFN-untreated	1		1	
Non-SVR	0.86 (0.59–1.24)	0.445	1.05 (0.71–1.54)	0.794
SVR	<0.01 (0–0.17)	<0.001	<0.01 (0–0.26)	<0.001
Variables at the development of HCC				
Age (≥60 years)	1.06 (0.72–1.63)	0.773	–	
Total bilirubin (≥1.0 mg/dl)	1.45 (1.04–2.01)	0.028	1.21 (0.82–1.76)	0.332
Albumin (<3.5 g/dl)	2.07 (1.49–2.89)	<0.001	1.70 (1.16–2.49)	0.007
Prothrombin time (<70%)	1.44 (0.99–2.06)	0.059	0.97 (0.65–1.43)	0.874
ALT (≥40 IU/L)	1.12 (0.78–1.67)	0.531	–	
Platelet count ($<10 \times 10^4$ μl)	1.72 (1.23–2.41)	0.001	1.35 (0.93–1.96)	0.118
Tumor-related variables				
Number of tumors (multiple vs. single)	1.59 (1.10–2.26)	0.014	1.71 (1.16–2.46)	0.007
Size of largest tumor (≥20 mm)	1.15 (0.83–1.60)	0.395	–	
AFP (≥100 ng/ml)	1.71 (1.17–2.45)	0.006	1.50 (1.00–2.18)	0.047
DCP (≥40 mAU/ml)	1.33 (0.91–1.98)	0.145	–	
Initial treatment of HCC				
PTA/surgical resection	1.69 (1.16–2.53)	0.006	1.03 (0.68–1.60)	0.882

HCC hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

HCC may already be at a "carcinogenic stage" and have a higher potential to develop intrahepatic multicentric carcinogenesis than those without HCC [15]. Patients who have already developed HCC may have background features such as greater age and impaired liver function because of more advanced fibrosis. Therefore, the observed recurrence and survival rates in the present study are those of selected patients who were already at the carcinogenic stage, and are thus biased in comparison to previous observations on primary prevention of HCC development in patients who had received IFN therapy. Recently, Imai et al. reported that an inhibitory effect of IFN therapy on development of HCC in older patients was limited to patients with SVR [44]. This also supports the notion that patients already at a carcinogenic stage or with risk factors associated with HCC development, such as greater age or advanced fibrosis, require eradication of HCV infection in order to achieve a significantly better prognosis.

The present observation highlights the importance of eradication of HCV in order to prevent HCC recurrence and to achieve better survival in this patient group. Plenty of reports are available that demonstrated the favorable effect of IFN therapy on the recurrence of HCC and survival particularly in patients who achieved SVR [21–28]. Therefore, re-treatment with more potent IFN therapies, such as combination therapy of PEGylated IFN plus ribavirin [45], should be recommended for patients who previously underwent IFN treatment without achieving SVR.

The present study has limitations as it is retrospective in nature, and thus, patients enrolled were biased in favor of experience of IFN treatment, and also HCC patients with previous IFN treatment were a selected population from a large cohort of patients who had undergone IFN treatment. Also, information on the histological data that may have influence on the outcome of HCC patients was not available in the present study. Further prospective studies are required to address these issues.

In conclusion, the present study demonstrated that patients with SVR to IFN treatment before the development of HCV-related HCC showed lower second HCC recurrence rates and higher survival rates than those with non-SVR to previous IFN treatment or IFN-untreated patients. Therefore, treatment with potent antiviral therapy is recommended for patients in the latter groups in order to suppress recurrence and improve survival by eradicating HCV infection.

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