Table 4 Efficacy of transcatheter arterial chemotherapy using platinum analogue for advanced HCC in 152 patients unresponsive to TACE-epirubicin, according to type of therapy

	CR (%)	PR (%)	SD (%)	PD (%)
Total ($n = 152$)	6 (4%)	28 (18%)	35 (23%)	83 (55%)
Type of therapy				
HAI ($n = 73$)	1 (1%)	9 (12%)	16 (22%)	47 (65%)
CL ($n = 20$)	1 (5%)	3 (15%)	7 (35%)	9 (45%)
TACE ($n = 59$)	4 (7%)	16 (27%)	12 (20%)	27 (46%)

CL, chemolipiodalization; CR, complete remission; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; PD, progressive disease; PR, partial response; SD, stable disease; TACE, transcatheter arterial chemoembolization.

(1%) patients showed CR, 9 of 73 (12%) patients showed PR, 16 of 73 (22%) patients showed SD, and 47 of 73 (65%) patients showed PD; in CL group: 1 of 20 (5%) patients showed CR, 3 of 20 (15%) patients showed PR, 7 of 20 (35%) patients showed SD, and 9 of 20 (45%) patients showed PD; in TACE group: 4 of 59 (7%) patients showed CR, 16 of 59 (27%) patients showed PR, 12 of 59 (20%) patients showed SD, and 27 of 59 (46%) patients showed PD (Table 4).

Factor influencing curative effect (PR or CR)

We then investigated the factors associated with curative effect (PR or CR) after treatment using platinum analogue. Univariate analysis identified the following 11 factors that influenced the rate of curative effect (PR or CR): serum DCP (< 100 IU/L/≥ 100 IU/L, $P = 0.001$), serum AFP (< 200 μg/L/≥ 200 μg/L, $P = 0.005$), ICG-R15 (< 30%/≥ 30%, $P = 0.005$), tumor size (< 20 mm/≥ 20 mm, $P = 0.011$), portal vein invasion (yes/no, $P = 0.014$), total bilirubin (< 1.5 mg/dL/≥ 1.5 mg/dL, $P = 0.018$), treatment method (HAI/CL/TACE, $P = 0.021$), type of platinum analogue (carboplatin/cisplatin, $P = 0.021$), intrahepatic multiplicity that extended to both lobes (yes/no, $P = 0.028$), age (< 60/≥ 60, $P = 0.057$), and serum AST (< 50 IU/L/dL/≥ 50 IU/L, $P = 0.057$). These parameters were entered into multivariate logistic regression analysis. The curative effect (PR or CR) was significantly higher for elderly patients (aged ≥ 60, risk ratio: 7.75; 95% CI: 1.80–33.40), small size HCC (< 20 mm, risk ratio: 4.88; 95% CI: 1.62–14.71), TACE-platinum analogue treatment (yes, risk ratio: 3.91; 95% CI: 1.34–11.38), lower serum total bilirubin level (< 1.5 mg/dL, risk ratio: 3.44; 95% CI: 1.22–9.71), and Tumor multiplicity, extended to both lobes (no, risk ratio: 2.30 (1.03–7.09) (Table 5).

Table 5 Factors associated with curative effects in patients who underwent transcatheter arterial platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin

Factors	Category	Risk Ratio (95% confidence interval)	*P*
Age (year)	1: < 60	1	
	2: ≥ 60	7.75 (1.80–33.40)	0.006
Tumor size (mm)	1: ≥ 20	1	
	2: < 20	4.88 (1.62–14.71)	0.005
Tumor therapy	1: HAI	1	
	2: CL	2.47 (0.52–11.69)	0.256
	3: TACE	3.91 (1.34–11.38)	0.012
Bilirubin (mg/dL)	1: ≥ 1.5	1	
	2: < 1.5	3.44 (1.22–9.71)	0.020
Multiple HCC, extended to both lobes (yes/no)	1: yes	1	
	2: no	2.30 (1.03–7.09)	0.044

CL, chemolipiodalization; CR, complete remission; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; PR, partial response; TACE, transcatheter arterial chemoembolization.

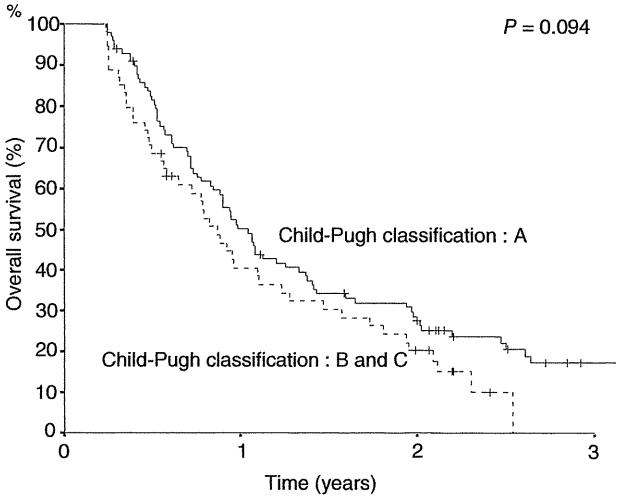

Figure 1 Cumulative survival rates after the first use of platinum-analogue for advanced HCC unresponsive to TACE-epirubicin, according to Child-Pugh classification.

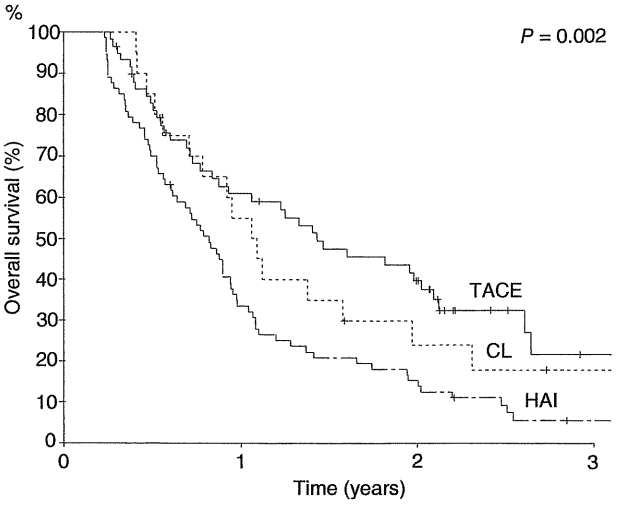

Figure 2 Cumulative survival rates after the first use of platinum-analogue for advanced HCC unresponsive to TACE-epirubicin, according to type of therapy.

Cumulative survival rate according to Child-Pugh classification

During the observation period, 125 of 152 (82.2%) patients died. The cumulative survival rates after the first use of platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin according to Child-Pugh classification were 50.1% at the end of the first year, 28.7% at the second year, and 17.2% at the third year for patients with Child-Pugh class A, and 40.5% at the first year, 20.3% at the second year, 0% at the third year for patients with Child-Pugh class B and C. The cumulative survival rates were slightly higher in patients with Child-Pugh class A than in those with Child-Pugh class B and C ($P = 0.094$), but no statistical significance was not there (Fig. 1).

Cumulative survival rate according to type of therapy

The cumulative survival rates after the first use of platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin according to type of therapy were 33.6% at the end of the first year, 15.4% at the second year, and 5.6% at the third year for patients with HAI group, 55.0% at the first year, 24.0% at the second year, 18.0% at the third year for patients with CL group, and 60.8% at the first year, 40.0% at the second year, 21.7% at the third year for patients with TACE group.

The cumulative survival rate was significantly different in these three type of therapy ($P = 0.002$) (Fig. 2).

Cumulative survival rate according to treatment effect

The cumulative survival rates after the first use of platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin were 81.8% at the end of the first year, 53.9% at the second year, and 33.1% at the third year for patients with PR or CR, and 36.6% at the first year, 17.5% at the second year, 7.4% at the third year for patients with PD or SD. The cumulative survival rates were significantly higher in patients with PR or CR than in those with SD or PD by transcatheter arterial chemotherapy using platinum analogue ($P < 0.001$) (Fig. 3). The 50% survival period was extended almost 1.4 year in patients with PR or CR by transcatheter arterial chemotherapy using platinum analogue.

Factor affecting survival rate

We then investigated the factors associated with survival rate after the first transcatheter arterial chemotherapy using platinum analogue for advanced HCC unresponsive to TACE-epirubicin. Univariate analysis identified the following 13 factors that influenced the survival rate: portal vein invasion (yes/no, $P < 0.001$), type of platinum analogue (carboplatin/cisplatin, $P = 0.001$), tumor size (< 20 mm/≥ 20 mm, $P = 0.001$), serum DCP (< 100 IU/L/≥ 100 IU/L, $P = 0.001$), age (< 60/≥ 60, $P = 0.001$), serum AFP (< 200 μg/L/≥ 200 μg/L, $P = 0.006$), treatment method (HAI/CL/TACE, $P = 0.008$), intrahepatic multiplicity that extended to both lobes

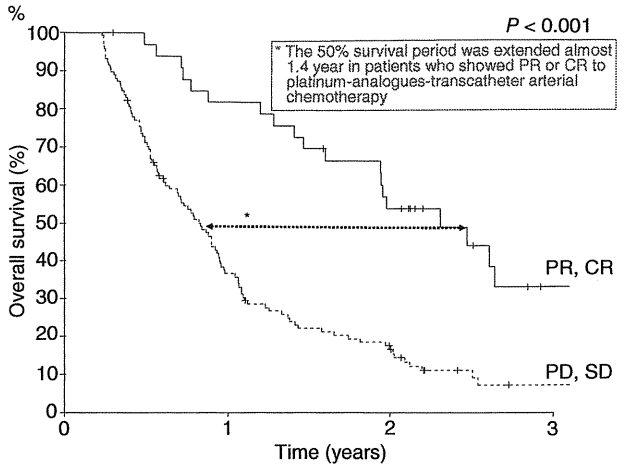

Figure 3 Overall survival rates after the first use of platinum-analogue for advanced HCC unresponsive to TACE-epirubicin, according to treatment effect.

(yes/no, $P = 0.012$), serum AST (< 50 IU/L/dL/ ≥ 50 IU/L, $P = 0.014$), prothrombin activity (< 80%/ ≥ 80%, $P = 0.020$), HBs-Ag (positive/negative, $P = 0.048$), anti HCV antibody (positive/negative, $P = 0.063$), and total bilirubin (< 1.5 mg/dL/≥ 1.5 mg/dL, $P = 0.068$). These parameters were entered into multivariate Cox proportional hazard analysis. The curative survival rate was significantly higher for small size HCC (< 20 mm, hazard ratio: 2.60; 95% CI: 1.30–5.19), no evidence of portal vein invasion (hazard ration: 2.08; 95% CI: 1.37–3.16), TACE treatment (yes, hazard ratio: 1.93; 95% CI: 1.23–3.02), HBs antigen negative (yes, hazard ratio: 1.82; 95% CI: 1.13–2.93), and low AST level (< 50 IU/L, hazard ratio: 1.67; 95% CI: 1.06–2.61) (Table 6).

Toxic effects

The most common side effects observed after treatment with a platinum analogue were fever and vomiting. Low-grade fever of 37–38°C lasting for a few days occurred in 78 (51.3%) patients, and fever of ≥ 38°C occurred in the other 51 (33.6%) patients. Nausea was reported in 125 (82.2%) patients, vomiting was noted at the time of treatment in 49 (32.2%) patients, dull pain in the upper abdomen was reported at the time of infusion by 31 patients (20.4%), a rise in serum aminotransferases was seen in 80 (52.6%) patients: 36 showed a rise up to twice the values before treatment, and 44 showed a rise of 1.5 to 1.9 times the baseline values. Within one week of treatment, 42 (27.6%) patients showed a transient rise in total bilirubin level to twice the pretreatment value. No adverse effects due to embolization in critical organs such as the lung, the heart, or brain were noted.

Causes of death

During the observation period, 125 (82.2%) patients died. The cause of death was HCC in 106 (84.8%) patients, liver failure in 14 (11.2%) patients and other causes in 5 (4.0%) patients.

Table 6 Factors associated with overall survival rate in patients with underwent transcatheter arterial platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin (Multivariate Cox proportional hazard analysis)

Factors	Category	Hazard Ratio (95% confidence interval)	*P*
Tumor size (mm)	1: ≥ 20	1	
	2: < 20	2.60 (1.30–5.19)	0.007
Portal vein invasion (yes/no)	1: yes	1	
	2: no	2.08 (1.37–3.16)	0.001
Tumor therapy	1: HAI	1	
	2: CL	1.80 (1.00–3.24)	0.050
	3: TACE	1.93 (1.23–3.02)	0.004
HBs antigen	1: positive	1	
	2: negative	1.82 (1.13–2.93)	0.013
AST (IU/L)	1: ≥ 50	1	
	2: < 50	1.67 (1.06–2.61)	0.026

CL, chemolipiodalization; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

DISCUSSION

ALTHOUGH TACE IS one of the most potent methods of treatment for unresectable HCC, various types of resistances to therapy can occur during the repetition of embolization. However, to our knowledge, there is no report that assessed the efficacy of platinum analogue for TACE-resistant HCC patients. Since various chemotherapeutic agents have occasionally produced objective tumor regression in palliative management of advanced liver cancer, we used platinum analogues for embolization-resistant HCC.

In our study, the 50% survival period was extended almost 1.4 year in patients who showed PR or CR in response to transcatheter arterial chemotherapy using platinum analogue for TACE-resistant HCC. We consider this outcome encouraging in TACE-resistant HCC, because these types of tumors are usually unexpected respond to any treatment. Multivariate analysis indicated that it is important to deliver the platinum analogue via TACE in order to achieve a satisfactory curative effect. A previous study reported the efficacy of cisplatin when injected with Lipiodol for preoperative TACE therapy (termed "sandwich therapy") in patients with HCC who received TACE as the first treatment.[14]

In univariate analysis, the cumulative survival rates were slightly higher in patients with Child-Pugh class A than in those with Child-Pugh class B and C, but in multivariate analysis, the factor of liver function was not significantly related to survival rate. In the result of multivariate analysis, tumor related factors more affected on the cumulative survival rates than liver function in this study.

The type of therapy, in univariate analysis, the cumulative survival rates were significantly different among the therapies. Multivariate analysis disclosed that the type of therapy significantly affected on cumulative survival rate after the first use of platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin.

These results indicated that it is important to deliver the platinum analogue via TACE in order to obtain a more long term survival, as well as curative effect.

Our results showed that the survival rate of HBV-positive HCC patients was significantly lower than that of HCV-positive patients with advanced HCC. HBV-related HCC or non-viral hepatitis-related HCC are often diagnosed at a more advanced stage than HCV-related HCC, and patients with such advanced-stage HCC related to HBV or non-viral etiology showed poor prognosis compared to those with HCV-related HCC.[21] This may explain the lower survival rate in our patients with HBV-related HCC.

In this study, we used two types of platinum analogues (cisplatin and carboplatin), and the results of univariate analysis, the curative effect (PR and CR) and overall survival rate were significantly better for cisplatin than carboplatin use. It is reported that cisplatin is more effective as an anti-tumor agent compared with carboplatin,[22,23] although there are no studies that compared the efficacy of the two agents in HCC. Our study was limited because it was retrospective in nature, but our results indicate that the chemotherapeutic effects of platinum analogues against HCC are more favorable for cisplatin than carboplatin, similar to other solid tumors. Further studies are required to investigate the underlying mechanism for the difference in efficacy of cisplatin than carboplatin.

A number of molecular-based chemotherapeutic agents are expected to become available in the future, such as Sorafenib,[24] and the primary therapy of advanced stage HCC may change with the introduction of these drugs. However, the results of our study suggested the advantage of using cisplatin in patients with TACE-resistant HCC. Although further studies are required to confirm our findings, the combination of various types of molecular targeting drugs and TACE may improve the treatment outcome in advanced-stage HCC.

In conclusion, the present study reports the efficacy of platinum analogues in patients with advanced HCC unresponsive to TACE-epirubicin. Most such patients have poor prognosis mainly because of lack of effective therapy. However, our results show that the 50% survival period of patients who respond to platinum analogues-transcatheter arterial chemotherapy was extended to almost 1.4 years. Accordingly, we recommend this form of chemotherapy for patients with advanced HCC unresponsive to TACE-epirubicin.

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High Serum Des-gamma-carboxy Prothrombin Level Predicts Poor Prognosis After Radiofrequency Ablation of Hepatocellular Carcinoma

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BACKGROUND: Currently, surgical resection is considered the first-line treatment for early stage hepatocellular carcinoma (HCC). Radiofrequency ablation (RFA) has been an alternative choice for unresectable HCC. However, RFA is expected to have similar therapeutic efficacy for early stage HCC with fewer invasions. **METHODS:** The authors retrospectively analyzed 199 patients who underwent surgery and 209 patients who underwent RFA for HCC with a maximum diameter of ≤3 cm and tumors numbering ≤3. All patients were complicated with Child-Pugh A cirrhosis. **RESULTS:** The 3- and 5-year survival rates of the resection (90.3%, 79.0%, respectively) and RFA groups were similar (87.4%, 74.8). The 1- and 3-year tumor recurrence-free survival rates of the resection group (83.1%, 51.0%, respectively) were higher than in the RFA group (82.7%, 41.8%; $P=.011$). Multivariate analysis identified prothrombin time ≥80% (hazard ratio [HR], 2.72; 95% confidence interval [CI], 1.56-4.74; $P < .001$) as an independent prognostic factor for survival in the resection group. Des-gamma-carboxy prothrombin (DCP) <100 arbitrary units (AU)/L (HR, 5.49; CI, 2.23-13.5; $P < .001$) and platelet count $\geq 1.0 \times 10^5$ (HR, 2.70; CI, 1.24-5.88; $P=.012$) were significant markers in the RFA group. Among patients with DCP ≥100 AU/L, treatment procedure (HR, 1.26; CI, 1.04-1.53; $P=.020$) was a significant prognostic factor for survival. **CONCLUSIONS:** High DCP levels reflect the biologic aggressiveness and progression of HCC tumors. In the aforementioned cases, we recommend surgical resection rather than RFA for such patients. **Cancer 2009;115:571-80. © 2008 American Cancer Society.**

KEY WORDS: hepatocellular carcinoma, DCP, radiofrequency, prognostic factor.

Hepatocellular carcinoma (HCC) is the sixth most common malignancy and third most deadly carcinoma in the world.[1] In Japan, HCC is ranked third among males and fifth among females as the leading causes of cancer death.[2] Most patients with HCC are infected with either hepatitis B virus (HBV) or hepatitis C virus (HCV), and have complications stemming from underlying chronic liver disease. The importance of liver condition in the treatment of HCC should be clearly discerned.[3]

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A maximum tumor diameter of ≤3 cm and tumors numbering ≤3 are good candidates for liver transplantation in patients with Child-Pugh class B and C.[4,5] However, patients with Child-Pugh class A conditions should be treated curatively.[5,6] Hepatectomy is currently recommended for patients with single asymptomatic HCC and extremely well-preserved liver function, who have neither clinically substantial portal hypertension nor abnormal bilirubin levels.[6] However, resection is suitable for only 20% to 35% of patients with HCC because of poor hepatic reserve.[7,8] Radiofrequency ablation (RFA) was introduced as a minimally invasive therapy for such cirrhotic patients.[9-13] RFA was the initial choice for unresectable HCC; however, 2 recent randomized controlled trials concluded that there were no substantial statistical survival differences between resection and RFA.[9,10] Although the results of these studies have not yet reached a worldwide consensus, some authors recommend RFA as a first-line therapy for such early stage HCC.[11-13]

Tumor staging and the decision between possible treatment options are conducted predominantly based on tumor size, number, vascular invasion, and extrahepatic metastasis evaluated by imaging analysis such as ultrasonography or dynamic computed tomography (CT). However, the malignant nature of the tumor as well as other characteristics are not generally considered.[14,15] Alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) are HCC-specific tumor markers. High levels of serum tumor markers often indicate HCC development in the liver. On the basis of histopathological analysis, serum AFP and DCP levels are also correlated with tumor differentiation, microscopic portal invasion, or intrahepatic metastasis.[16,17]

This present study is an attempt to evoke discussion on treatment strategies for small HCC measuring ≤3 cm by comparing the long-term outcome of patients treated with either hepatectomy or RFA as the first-line treatment for HCC. AFP and DCP were also accounted as indicators in the decision-making and treatment procedure.

MATERIALS AND METHODS

Patients

A total of 1057 patients were admitted to the Department of Hepatology, Toranomon Hospital between 1995 and 2006 for the treatment of initially developed HCC. The major background liver disease was HCV (767 patients, 72.6%), followed by HBV (196 patients, 18.5%), HCV + HBV (8 patients, 0.8%), alcoholic liver diseases (habitual drinking of ethanol at >80 g/day, 48 patients, 4.5%), primary biliary cirrhosis (4 patients, 0.4%), autoimmune hepatitis (2 patients, 0.2%), and cryptogenic liver disease (42 patients, 4.0%). Treatment of HCC included surgical resection in 281 patients, local ablation therapy in 398 patients (RFA, 267 patients; microwave coagulation, 47 patients; ethanol injection, 84 patients), and transarterial chemoembolization in 378 patients. Among these patients, we included patients with Child-Pugh A cirrhosis and HCC measuring ≤3 cm in diameter and numbering ≤3 tumors who were treated radically by either surgical resection or RFA. Table 1 summarizes the profile of the 199 patients who received resection and 209 patients who received RFA. HBV-related liver diseases were more common among patients who underwent resection, who were younger (62 vs 67 years; $P < .001$) than patients with RFA. The maximum tumor diameter was larger in the resection group than in the RFA group (20 vs 18 mm; $P < .001$). With regard to laboratory tests, serum albumin level, platelet count, and prothrombin time (%) were higher among patients in the resection group, whereas serum aspartate aminotransferase (AST) levels were higher among patients in the RFA group. None of the patients in either group had tumor invasion of the major portal branch or extrahepatic metastasis. Our institution does not require informed consent for retrospective analysis.

Diagnosis of HCC

Diagnosis of HCC was predominantly based on image analysis. If a hepatic nodular lesion was found on screening ultrasonography, the patient underwent dynamic CT and/or dynamic magnetic resonance imaging (MRI). Furthermore, when a liver nodule showed hyperattenuation in the arterial phase of dynamic study and washout in portal or delayed phase, or showed typical hypervascular staining on digital subtraction angiography, the nodule was diagnosed as HCC. According to the American Association for the Study of Liver Disease guidelines, we obtained at least 2 dynamic imaging images before treatment.[5] When the nodule did not appear in the abovementioned typical imaging features, a fine needle aspiration biopsy was carried out followed by histological examination and diagnosis.

Table 1. Clinical Background of 199 Patients Who Underwent Hepatic Resection and 209 Patients Who Underwent Radiofrequency Ablation of Liver Tumor

Factors	Resection Group (n = 199)	RFA Group (n = 209)	*P*
Age, y*	62 (29-80)	67 (38-87)	<.001
Sex, men:women	146:53	137:72	NS
HBV:HCV:HBV + HCV:others	60:121:3:15	22:176:1:10	<.001
Habitual alcohol intake, yes:no	29:170	9:200	
Diameter of HCC, mm*	20 (9-30)	18 (8-30)	<.001
No. of HCC, 1:2:3	168:22:9	169:29:11	
Tumor vascularity, present:absent	185:14	156:53	
Albumin, g/dL*	3.7 (2.8-4.7)	3.6 (2.6-4.4)	<.001
Bilirubin, mg/dL*	1.0 (0.3-2.4)	1.0 (0.2-2.4)	NS
AST, IU/L*	43 (13-386)	55 (17-208)	<.001
Platelets, $\times 10^4/mm^{3*}$	13.1 (4.0-27.2)	10.5 (2.7-25.3)	<.001
Prothrombin time, %*	92 (62-115)	88 (57-125)	.006
AFP, ng/mL*	22 (1-7960)	18 (2-1490)	NS
DCP, AU/L*	20 (<10-1650)	17 (<10-1370)	NS

RFA indicates radiofrequency ablation; NS, not significant; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; IU, international units; AFP, alpha-fetoprotein (AFP); DCP, des-gamma-carboxy prothrombin; AU, arbitrary units.
*Data are expressed as median (range).

Method of Treatment

Physicians and surgeons usually discuss together the preferred choice of therapy in individual patients. Hepatic resection was performed under intraoperative ultrasonographic monitoring and guidance. For small and superficial HCCs, arterial and portal vein clamping at hepatic hilum was not usually performed to maintain liver perfusion.

RFA was performed using 3 different devices: the radiofrequency interstitial tumor ablation system (RITA, RITA Medical Systems Inc., Mountain View, Calif), the cool-tip system (Tyco Healthcare Group LP, Burlington, Vt), and the radiofrequency tumor coagulation system (RTC system, Boston-Scientific Japan Co., Tokyo, Japan). In the first 2 systems, treatment procedures were performed according to the protocol advised by the manufacturer. However, treatment using the RTC system was performed by adopting the "stepwise hook extension technique."[18] The needle was inserted into the tumor percutaneously under ultrasonographic guidance. Because the HCC nodule could not be observed by ultrasonography in 6 cases, the needle was inserted under CT assistance. In the case of RFA, dynamic CT was performed 1 to 3 days after therapy, and the ablated area was evaluated. The goal of treatment was to obtain a necrotic area larger than the original tumor size, with a surrounding treatment margin of $\geq$5 mm in all directions. When this was not achieved or a residual tumor was found, additional ablation was considered. Of 206 total patients, total ablation session was required once in 149 (71.3%) patients, twice in 47 (22.5%) patients, and 3 times or more in 13 patients (6.2%).

Measurement of Serum AFP and DCP

Serum AFP level was measured by chemiluminescent enzyme immunoassay (CLEIA) using a commercial assay kit (Lumipulse Prestoll AFP, Fujirebio Inc., Tokyo, Japan). DCP level was measured by CLEIA (Lumipulse PIVKA II Eisai, Eisai, Tokyo, Japan).

Follow-up Protocol

Physicians examined the patients every 4 weeks after treatment, and liver function tests and tumor markers were also measured once every month. After completion of HCC eradication, recurrence was surveyed with contrast enhancement 3-phase CT every 3 months. Local tumor progression was defined as tumor recurrence adjacent to the resected or ablated area.

Statistical Analysis

Differences in background features and laboratory data between resection and RFA groups were analyzed by the chi-square test and Mann-Whitney *U* test. Survival and recurrence-free survival were analyzed using the Kaplan-Meier technique, and differences in curves were tested using the log-rank test. Independent risk factors associated with survival and recurrence-free survival were studied using stepwise Cox regression analysis.[19] Potential risk factors for survival and recurrence-free survival included the following 14 variables: age, sex, etiology of background liver disease, amount of alcohol intake, serum albumin, bilirubin, AST, platelet count, prothrombin time, AFP, DCP, diameter of the HCC, tumor multiplicity, and tumor vascularity evaluated by dynamic CT or dynamic MRI. A probability of less than .05 was considered significant. Data analysis was performed using SPSS statistical software version 10 (SPSS Inc., Chicago, Ill).

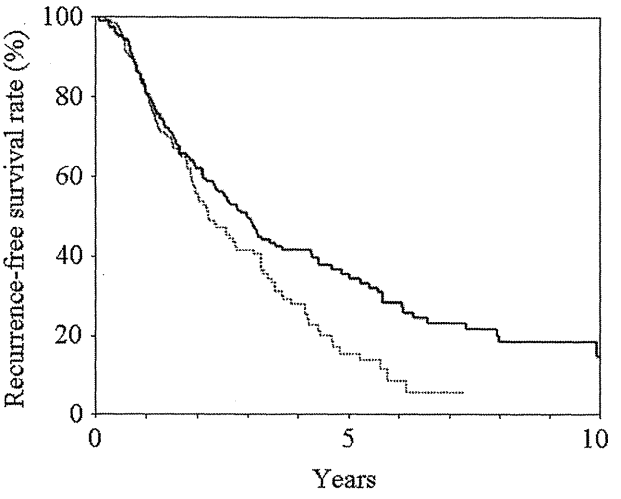

FIGURE 1. Cumulative recurrence free survival rates of the patients who underwent surgical resection (solid line) and radiofrequency ablation (RFA) (dotted line) are shown. The recurrence-free survival rate of the resection group was higher than of the RFA group ($P=.011$).

RESULTS

Survival and Recurrence-free Survival Rates

During the median follow-up of 3.3 years (range, 0.1-12.2 years), 112 (56.3%) of 199 patients in the resection group and 120 (57.4%) of 209 patients in the RFA group developed HCC recurrence. HCC recurrences mainly occurred in other sites in the liver. However, in the RFA group, local tumor progression, defined as HCC recurrence adjacent to the treated site, was seen in 18 (8.6%) of 209 patients, but noted in only 1 patient of the resected group. The cumulative local tumor progression rate in the RFA group was 2.7%, 11.3%, and 12.5% at 1, 3, and 5 years, respectively. The tumors were treated with surgical resection in 2 patients, additional tumor ablation in 8 patients, and transcatheter chemoembolization in the remaining 8 patients.

Exactly 64 patients of the resection group and 31 patients of the RFA group died during the follow-up. The cause of death among patients in the resection group was tumor progression in 51, hepatic failure in 10, gastrointestinal bleeding in 1, and other causes in 2. Uniformly, the cause of death in the RFA group was tumor progression in 16, hepatic failure in 13, gastrointestinal bleeding in 1, and other causes in 1. The cumulative survival rates for the resection group at 1, 3, 5, and 7 years were 96.9%, 90.3%, 79.0%, and 61.5%, whereas those for the RFA group were 99.0%, 87.4%, 74.8%, and 65.4%, respectively. The overall survival rates were not significantly different between the 2 groups.

The tumor recurrence-free survival rates for the resection group at 1, 3, 5, and 7 years were 83.1%, 51.0%, 36.8%, and 23.3%, and for the RFA group were 82.7%, 41.8%, 17.0%, and 5.8%, respectively (Fig. 1). The recurrence-free survival rate was higher in the resection group than in the RFA group ($P=.011$).

Factors Associated With Survival in Patients in the Resection Group

Among 199 patients treated with surgical resection, factors associated with survival were evaluated by both univariate and multivariate analysis (Table 2). Single tumor, serum albumin >3.8 g/dL, and prothrombin time >80% were significant by Kaplan-Meier analysis, whereas factors such as age, sex, etiology of background liver disease, amount of alcohol intake, bilirubin, AST, platelet count, AFP, DCP, diameter of the HCC, and tumor vascularity were not significantly related to patients' survival. In a multivariate analysis using the Cox proportional hazard model, prothrombin time >80 % (hazard ratio [HR], 2.72; 95% confidence interval [CI], 1.56-4.74; $P<.001$) was the independent prognostic factor for survival.

Table 2. Results of Univariate and Multivariate Analyses of Factors Associated With Survival of Patients Treated by Surgical Resection

Variable	No.	% 5-Year Survival Rate	Hazard Ratio (95% CI)	*P*
Univariate analysis				
No. of tumors				.012
Single	167	82.4	–	
Multiple	32	59.8	–	
Albumin, g/dL				.020
≥3.8	134	91.1	–	
<3.8	64	73.1	–	
Prothrombin time (%)				.003
≥80	168	83.0	–	
<80	29	60.1	–	
Multivariate analysis				
Prothrombin time, %, ≥80/<80			2.72 (1.56-4.74)	<.001

CI indicates confidence interval.

Table 3. Results of Univariate and Multivariate Analyses of Factors Associated With Survival in Patients Treated With Radiofrequency Ablation

Variable	No.	% 5-Year Survival Rate	Hazard Ratio (95% CI)	*P*
Univariate analysis				
Platelets, $\times 10^4/mm^3$				.006
≥10	111	84.1	–	
<10	98	65.5	–	
DCP, AU/mL				<.001
<100	187	77.3	–	
≥100	13	33.6	–	
Multivariate analysis				
DCP, AU/L, <100/≥100			5.49 (2.23-13.5)	<.001
Platelets, $\times 10^4/mm^3$, ≥10/<1			2.70 (1.24-5.88)	.012

CI indicates confidence interval; DCP, des-gamma-carboxy prothrombin; AU, arbitrary units.

Factors Associated With Survival in Patients in the RFA Group

We also evaluated the factors associated with the survival of 209 patients treated with RFA (Table 3). Platelet count $\geq 1.0 \times 10^5$ and DCP <100 arbitrary units (AU)/L were significant in univariate analysis, whereas 12 other variables were not associated with survival. Multivariate analysis identified DCP <100 AU/L (HR, 5.49; 95% CI, 2.23-13.5; $P < .001$) and platelet count $\geq 1.0 \times 10^5$ (HR, 2.70; 95% CI, 1.24-5.88; $P = .012$) as significant and independent determinants of survival.

Factors Associated With Recurrence-free Survival in Patients in the Resection Group

Next, we evaluated the factors associated with recurrence-free survival in patients treated with surgical resection (Table 4). Presence of a single tumor, serum albumin ≥3.8 g/dL, platelet count $\geq 1.0 \times 10^5$, and prothrombin time ≥80% were significant in the univariate analysis, whereas 10 other variables were not significant factors for recurrence-free survival. In multivariate analysis, single tumor (HR, 2.39; 95% CI, 1.51-3.80; $P < .001$), serum albumin ≥3.8 g/dL (HR, 1.54; 95% CI, 1.02-2.32;

Table 4. Results of Univariate and Multivariate Analyses of Factors Associated With Recurrence-free Survival Among Patients Treated With Resection

Variable	No.	% 3-Year Survival Rate	Hazard Ratio (95% CI)	*P*
Univariate analysis				
No. of tumors				<.001
Single	166	55.4	–	
Multiple	32	24.5	–	
Albumin, g/dL				.009
≥3.8	63	71.7	–	
<3.8	134	42.1	–	
Platelets, $\times 10^4/mm^3$				.025
≥10	137	60.3	–	
<10	60	33.0	–	
Prothrombin time, %				.009
≥80	167	55.7	–	
<80	29	29.0	–	
Multivariate analysis				
No. of tumors, single/multiple			2.39 (1.51-3.80)	<.001
Albumin, g/dL, ≥3.8/<3.8			1.54 (1.02-2.32)	.040
Platelets, $\times 10^4/mm^3$, ≥10/<10			1.47 (1.03-2.12)	.036

CI indicates confidence interval.

$P=.040$), and platelet count $\geq 1.0 \times 10^5$ (HR, 1.47; 95% CI, 1.03-2.12; $P=.036$) were independent prognostic factors for recurrence-free survival.

Factors Associated With Recurrence-free Survival in Patients in the RFA Group

Factors associated with recurrence-free survival were evaluated in patients treated by RFA. The 3-year recurrence-free survival rate was 44.7% in 185 patients with DCP <100AU/L, whereas it was 0.0% in 13 patients with DCP ≥100 AU/L. Univariate and multivariate analysis identified only DCP <100 AU/L (HR, 6.82; 95% CI, 3.49-13.3; $P < .001$) as a significant determinant of recurrence-free survival.

Survival and Recurrence-free Survival in Patients With DCP >100 AU/L

Figure 2 shows the cumulative survival rate, and Figure 3 shows the recurrence-free survival rate based on DCP levels. The survival rate and recurrence-free survival rate were associated with DCP in the RFA group, but they were not associated with DCP in the resection group. AFP >400 AU/L was associated with neither survival rate nor recurrence-free survival rate in either the resection or the RFA group. Therefore, 27 selected patients from the resection group and 13 from the RFA group whose DCP was ≥100 AU/L were examined to determine whether the overall survival rate was different between the resection and RFA groups. The backgrounds of the 2 groups based on treatment procedure are shown in Table 5. Treatment procedure (resection), age <65 years and serum albumin >3.8 g/dL were significant in the univariate analysis. Multivariate analysis revealed that treatment procedure (HR, 1.26; 95% CI, 1.04-1.53; $P=.020$) was a significant and independent determinant in the overall survival rate (Table 6).

DISCUSSION

Patients with HCC usually have a history of chronic liver disease, especially cirrhosis. Unfortunately, even when curative therapy is performed, tumor recurrence is frequent. For this reason, less invasive treatment procedures are needed to preserve liver function.

The Barcelona Clinic Liver Cancer (BCLC) guideline for the treatment of HCC recommends resection for patients with a single HCC and Child-Pugh A who have no other complications.[5] The suggested option for RFA includes patients with multiple tumors and associated

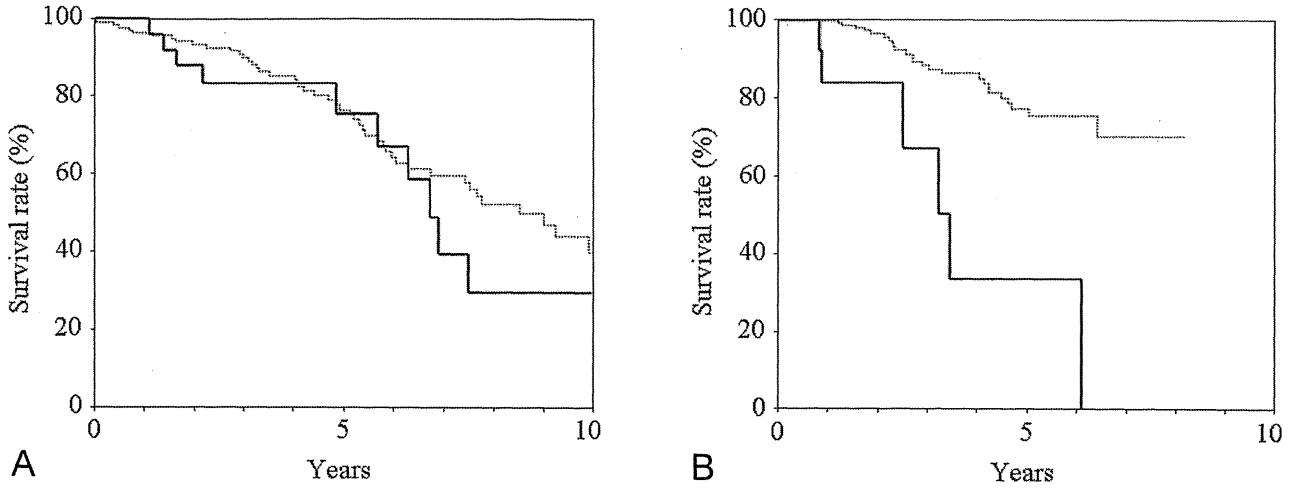

FIGURE 2. Cumulative overall survival rate of the patients who underwent surgical resection and radiofrequency ablation based on des-gamma-carboxy prothrombin (DCP) level is shown. Solid line indicates DCP level ≥100 AU/L; dotted line, DCP level <100 AU/L. (A) Cumulative survival rate of patients who underwent resection based on DCP level is shown. (B) Cumulative survival rate of patients who underwent radiofrequency ablation (RFA) based on DCP level is shown. Prognosis of patients who underwent RFA varied according to DCP level, whereas prognosis of patients who underwent resection was independent of DCP level.

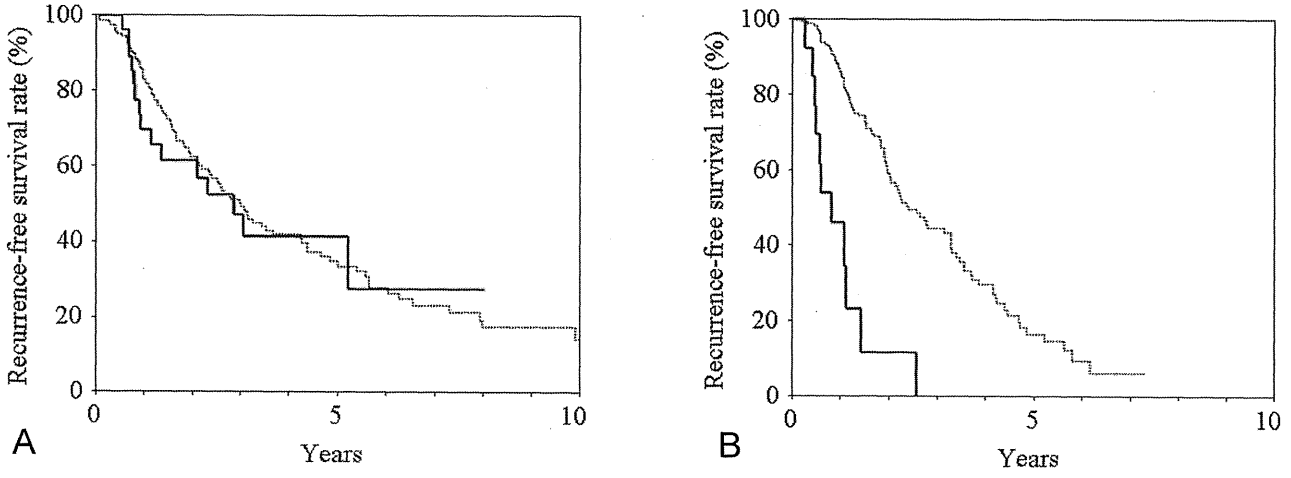

FIGURE 3. Cumulative recurrence-free survival (RFS) rate of the patients who underwent surgical resection and radiofrequency ablation based on des-gamma-carboxy prothrombin (DCP) level is shown. Solid line indicates DCP level ≥100 AU/L; dotted line, DCP level <100 AU/L. (A) Cumulative RFS rate of patients who underwent resection based on DCP level is shown. (B) Cumulative RFS rate of patients who underwent RFA based on DCP level is shown. Prognosis of patients who underwent RFA varied according to DCP level.

disease. However, in clinical practice, RFA is widely applied as a curative treatment for variable stages of HCC. Advances in imaging diagnosis have allowed identification of small HCC measuring <2 cm during the course of chronic liver disease.[3] The use of RFA seems to be an excellent option for the aforementioned tumors.

In the present study, we focused on the malignant potential of HCC and examined 2 representative tumor markers of HCC. AFP has been used as a tumor marker for HCC worldwide, and is considered by some as a predictor of survival or recurrence after RFA.[12] DCP is also useful as a prognostic factor in patients with HCC.[20-22]

In the present study, serum albumin levels and prothrombin time, which reflect liver function, were significantly associated with survival in patients who undergo resection similar to tumor multiplicity. Likewise, serum

Table 5. Clinical Background of 27 Patients Who Underwent Resection and 13 Patients Who Underwent Radiofrequency Ablation

Factors	Resection Group, n = 27	RFA Group, n = 13	*P*
Age, y*	60 (35-73)	67 (50-78)	.006
Sex (men:women)	23:4	10:3	NS
HBV:HCV:others	13:12:2	1:9:3	.015
Habitual alcohol intake, yes: no	3:24	2:11	NS
Diameter of HCC, mm*	22 (14-30)	22 (10-30)	NS
No. of HCC, single:multiple	26:1	9:4	.031
Tumor vascularity, present:absent	27:0	12:1	NS
Albumin, g/dL*	3.8 (3.3-4.3)	3.4 (2.6-4.1)	.003
Bilirubin, mg/dL*	0.9 (0.4-1.9)	0.9 (0.4-1.8)	NS
AST, IU/L*	38 (16-240)	49 (17-145)	NS
Platelets, $\times 10^4/mm^3$*	15.1 (6.0-24.5)	10.5 (4.5-24.6)	.025
Prothrombin time, %*	94 (79-112)	86 (73-110)	NS

* Data are expressed as median (range).

Table 6. Results of Univariate and Multivariate Analyses of Factors Associated With Survival Among Patients With Serum Des-gamma-carboxy Prothrombin Level ≥100

Variable	No.	% 5-Year Survival Rate	Hazard Ratio (95% CI)	*P*
Univariate analysis				
Age, y				.026
<65	25	87.7	—	
≥65	15	45.5	—	
Albumin, g/dL				.024
≥3.8	12	100	—	
<3.8	28	58.4	—	
Treatment procedure				.012
Resection	27	83.4	—	
RFA	13	33.6	—	
Multivariate analysis				
Treatment procedure				.020
Resection or RFA			1.26 (1.04-1.53)	

CI indicates confidence interval; RFA, radiofrequency ablation.

albumin level, platelet count, prothrombin time, and presence of multiple tumors were associated with recurrence-free survival. Alternatively, in RFA patients, in addition to platelet count, which indicates severity of portal hypertension, DCP levels were significant predictors of survival. Likewise, DCP levels were also significant predictors in recurrence-free survival. It is noteworthy that both survival and disease-free survival rates of patients who undergo RFA, but not resection, are correlated with DCP levels by multivariate analysis.

It is difficult to explain why DCP influenced survival and disease-free survival in the RFA group but not the resection group. We speculate that a high level of serum tumor marker reflects a high tumor malignant potential. Therefore, for a biologically aggressive tumor like HCC, resection is recommended over RFA because the radical nature of surgical resection may be superior to RFA.

According to previous reports, DCP is related to histological features of HCC.[21,22] Shirabe et al[21] examined

218 HCC patients who underwent surgical resection for HCC and concluded that serum DCP level is a predictor of microvascular invasion. They identified microvascular invasion in 44% of their patients with DCP ≥100 AU/L, but in only 16% of patients with DCP <100 AU/L. Shimada et al[22] examined explanted liver transplants and reported that serum DCP level is associated with vascular invasion and HCC recurrence. When we evaluated the relationship between clinicopathological features and serum DCP level in the resection group, microvascular invasion was found in 11 (44.0%) of 25 patients with DCP ≥100 AU/L, but in only 22 (13.6%) of 162 patients with DCP <100 AU/L, which was similar to the results in Shirabe et al.[21]

Unfortunately, in the RFA group, histological examination was performed only on nodules that showed atypical image findings. Moreover, it is sometimes difficult to judge microscopic vascular invasion in small specimens obtained by needle biopsy. However, we cannot deny the presence of microscopic vascular invasion in patients with high levels of tumor markers who have been treated with RFA. Furthermore, high levels of DCP are not only a marker of malignancy, but also indicate the biologic aggressiveness and progression of the HCC tumor. Hence, HCCs with high levels of DCP have greater chances of hypervascularity and early infiltration than do HCCs with lower levels of DCP.

In general, microscopic vascular invasion or intrahepatic metastasis is a poor prognostic factor for survival and recurrence-free survival even in patients who undergo surgical resection.[23-25] Why was survival and recurrence-free survival not different among the resection group in our study? One of the reasons is that we included patients with an HCC of a maximum diameter of ≤3 cm. In contrast, most previous studies included HCC as large as 5 cm in diameter.[23-25] The biological features of malignancy might be worse in such large tumors. We speculate that in HCC measuring ≤3 cm (median 2.0 cm), minimal microscopic vascular invasion or intrahepatic metastasis adjacent to the main tumor can be curatively resected by surgery, whereas these sometimes become incompletely necrotic even when a sufficient surrounding margin is obtained from treatment with RFA.

Analysis of factors associated with survival in patients with DCP of ≥100 AU/L showed that the type of treatment procedure (eg, hepatectomy) significantly influenced outcome. In contrast, no such relationship was found in patients with DCP of <100 AU/L. These results indicate that DCP is an important factor in selecting treatment procedure for patients with HCC measuring ≤3 cm and numbering ≤3 tumors.

In conclusion, DCP levels were significant predictors of both survival and recurrence-free survival in the RFA group. Hence, when the level of DCP is high, hepatic resection should be the treatment of choice even if the maximum tumor diameter is ≤3 cm and there are ≤3 tumors. If the level is low, RFA should be considered, because it is less invasive.

Because the current study was retrospective in nature, it has certain limitations and potential biases. The baseline characteristics of the 2 groups were quite different. Although we enrolled only Child-Pugh A patients, the resection group was younger and had better liver function. However, etiology of the liver disease was also different; the overall survival rates were not significantly based on etiology in either the resection or the RFA group. Therefore, we believe that the etiology of liver disease could be ignored in these patients. Our study did not uncover the reason for the high risk of mortality and tumor recurrence in patients in the RFA group with high levels of DCP. A cohort validation study is needed to confirm our results. In addition, clinicopathological and molecular analyses are also needed to define the biological significance of the biomarker.

Conflict of Interest Disclosures

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An Open Pilot Study Exploring the Efficacy of Fluvastatin, Pegylated Interferon and Ribavirin in Patients with Hepatitis C Virus Genotype 1b in High Viral Loads

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Key Words
Hepatitis, chronic · Virus, hepatitis C · Statin · Interferon, pegylated · Ribavirin

Abstract
Objective: Response to pegylated (PEG) interferon (IFN) and ribavirin is achieved only in 40–50% of patients infected with hepatitis C virus (HCV) of genotype 1 in high viral loads, which needs to be improved. ***Methods:*** In an open-label pilot study, fluvastatin (HMG-CoA reductase inhibitor), 20 mg daily, was given along with PEG-IFN/ribavirin to 21 patients with chronic hepatitis C. They were followed for HCV RNA in serum. ***Results:*** During treatment for 48 weeks, HCV RNA was lost from serum in 93% of the patients. In the 15 patients who received 48-week therapy, a sustained virological response (SVR) with loss of HCV RNA 24 weeks after completion was achieved in 10 (67%), including 7 of the 9 (78%) male and 3 of the 6 (50%) female patients. In the remaining 6 patients who received 72-week therapy, SVR was gained in 4 (67%), including 1 of the 2 male and 3 of the 4 female patients aged 56, 58 and 62 years, respectively. ***Conclusion:*** Fluvastatin could be used safely to increase the response to PEG-IFN and ribavirin, especially in aged women who respond poorly to combined PEG-IFN/ribavirin.

Introduction

Over the world approximately 190 million people are persistently infected with hepatitis C virus (HCV) [1], and about 30% of them develop serious liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC) [2]. Interferon (IFN) is the only drug that can resolve HCV infection. However, using the most advanced treatment currently available, with pegylated (PEG)-IFN and ribavirin for 24–48 weeks, a sustained virological response (SVR) defined by the loss of HCV RNA from serum 24 weeks after completion of therapy is achieved in 40–80% of the patients [3–5]. The response rate is influenced by host factors such as sex, age and ethnicity [6–8], as well as virological factors including genotypes and viral loads [9]. It remains unsatisfactorily low in patients infected with HCV genotype 1 at merely 40–50%, and dismal in women aged 50 years or older at merely 22% [10]. Hence, there is an imminent need to improve the efficacy of antiviral treatment for terminating HCV infection in these patients.

Efforts have been devoted toward increasing the efficacy to PEG-IFN/ribavirin therapy. Inhibitors of viral protease, alone or in combination with the IFN-based treatment, have been found effective in preliminary studies [11, 12]. Recently, drugs that can inhibit the key enzyme

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for controlling the synthesis of cholesterol, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, have gained attention due to their potential to decrease the replication of HCV in clinical and experimental settings [13, 14].

More than three quarters of Japanese patients are infected with HCV genotype 1b in high viral loads. They are much older than Western patients owing to the widespread HCV infection which struck Japan about 20 years ago [15]. Owing to this, the response to PEG-IFN/ribavirin in Japanese patients with chronic hepatitis C is poorer than that in Western countries. In an open-label pilot study, 21 patients with chronic hepatitis C, who were infected with HCV-1b at high viral loads, received triple treatment with fluvastatin, PEG-IFN and ribavirin. On-treatment viral dynamics and SVR achieved by the triple therapy could offer hope for improving the response in patients infected with HCV-1 in high viral loads.

Patients and Methods

From December 2005 to March 2006, 21 patients with chronic hepatitis C agreed to receive fluvastatin, in addition to standard PEG-IFN/ribavirin treatment, at the Department of Hepatology, Toranomon Hospital, Metropolitan Tokyo. They all were: (1) positive for antibody to HCV (anti-HCV) and HCV RNA of genotype 1b, and not co-infected with HCV of the other genotypes; (2) negative for hepatitis B surface antigen or antibody to human immunodeficiency virus type-1 (HIV-1); (3) confirmed within the past 2 months to have high HCV RNA levels of ≥100 KIU/ml, which is the Japanese definition of high viral loads [16, 17]; (4) platelet counts >80 × 10^3/mm^3 and without cirrhosis diagnosed by ultrasonography; (5) body weight >40 kg and not pregnant or lactating; (6) a total alcohol intake of <500 kg during the past; (7) without HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune hepatitis; (8) without antiviral or immunosuppressive treatment during the previous 3 months, and (9) with the wish to comply to the treatment protocol for 48–72 weeks.

They were followed for liver function and virological markers at least monthly during treatment and until 24 weeks after completion of triple treatment. Informed consent was obtained from each patient, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Markers of HCV Infection

Anti-HCV was determined by third-generation enzyme-linked immunosorbent assay (ELISA) using commercial kits (Ortho HCV Ab ELISA Test 3; Chiron Cooperation, Emeryville, Calif., USA). HCV RNA was determined quantitatively by polymerase chain reaction (PCR; Cobas Amplicor HCV Monitor ver. 2.0, Roche Diagnostics, Tokyo, Japan), with a dynamic range from 5 to 5,000 KIU/ml, in sera diluted 10-fold at baseline, as well as every 2 weeks until 8 weeks after initiation of therapy and monthly thereafter. Sera negative for HCV RNA (<5 KIU/ml) by the quantitative assay were tested by qualitative PCR (Amplicor, Roche Molecular Systems, Inc., Branchburg, N.J., USA) with the detection limit at 100 copies/ml.

Fluvastatin Added to Combined PEG-IFN and Ribavirin

Patients received subcutaneous PEG-IFN-α2b (PEG-Intron, Schering-Plough Corp., Kenilworth, N.J., USA) weekly at a median dose of 1.5 (range 1.3–1.7) μg/kg, along with oral ribavirin daily at a median dose of 12.3 (range 10.2–13.7) mg/kg for 48–72 weeks. The dose of ribavirin was adjusted according to body weight: 600 mg for patients weighing ≤60 kg; 800 mg for those between >60 and <80 kg, and 1,000 mg for those ≥80 kg. In addition, the patients received 20 mg oral fluvastatin daily for 48–72 weeks.

Results

Patients Who Received Triple Treatment with Fluvastatin, PEG-IFN and Ribavirin

The baseline characteristics of the 21 patients infected with HCV-1b at a high viral load and who received triple therapy with fluvastatin, PEG-IFN and ribavirin are listed in table 1. Their median age was 56 years, and 11 (52%) were men. Their hematological and biochemical values including serum lipids were within normal limits, except for elevated levels of AST and ALT. A lack of mutations affecting the response to IFN in the core protein, i.e., mutations at positions 70 and 91 [16], and amino acid substitutions in the IFN-sensitivity determining region [18] were detected in HCV RNA from 29 and 35% of the patients.

Loss of HCV RNA from Serum in Patients with Triple Treatment

Figure 1 illustrates the cumulative loss of HCV RNA from the serum in 21 patients infected with HCV-1b at high loads. HCV RNA was cleared in 52% of the patients during the first 12 weeks. It decreased slowly thereafter, and was lost in 93% of the patients at 48 weeks. Triple treatment was continued in 6 of the 21 patients for an additional 24 weeks. HCV RNA was cleared from the serum in 5 (83%) of them at completion of the 72-week treatment.

Sustained Virological Response in Patients Receiving Triple Treatment

Of the 21 patients given triple treatment, 19 received it for 48 weeks. Skin rashes and general malaise developed in 2 of them at weeks 17 and 24, respectively, and fluvastatin was withdrawn while PEG-IFN and ribavirin were continued. Six patients continued to receive triple treatment for an additional 24 weeks. Therefore, the loss of HCV RNA from the serum 24 weeks after completion of

Table 1. Baseline characteristics of patients (n = 21) with chronic hepatitis C who received triple treatment with fluvastatin, PEG-IFN and ribavirin

	Normal ranges	Patients
Age, years	NA	56 (32–63)
Men	NA	11 (52%)
Albumin, g/dl	3.9–5.2	3.8 (3.4–4.2)
Hemoglobin, g/dl	11.3–17.0	14.4 (12.1–16.4)
Platelets, $\times 10^3/mm^3$	141–350	190 (119–240)
AST, IU/l	13–33	50 (21–208)
ALT, IU/l	6–42	64 (23–391)
γ-GTP	9–109	36 (11–301)
ICG_{15}, %	<10	12 (2–27)
HCV RNA, KIU/ml	NA	2,000 (14– >5,000)
Past treatments	NA	8 (36%)
Ribavirin dose, mg/kg	NA	12.3 (10.2–13.7)
Serum lipids		
Total cholesterol, mg/dl	122–240	163 (124–273)
LDL-C, mg/dl	86–160	99 (59–187)
HDL-C, mg/dl	35–75	46 (28–73)
Mutations in HCV RNA		
Wild-type at aa70	NA	12 (57%)
Wild-type at aa91	NA	10 (48%)
Double wild, aa71/aa90	NA	6 (29%)
Mutations in ISDR	NA	6/17 (35%)

Data are expressed as the number of patients with percentages in parentheses or median values with ranges in parentheses. AST = Aspartate aminotransferase; ALT = alanine aminotransferase; γ-GTP = γ-glutamyltranspeptidase; ICG_{15} = retention of indocyanine green at 15 min; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; NA = not applicable.

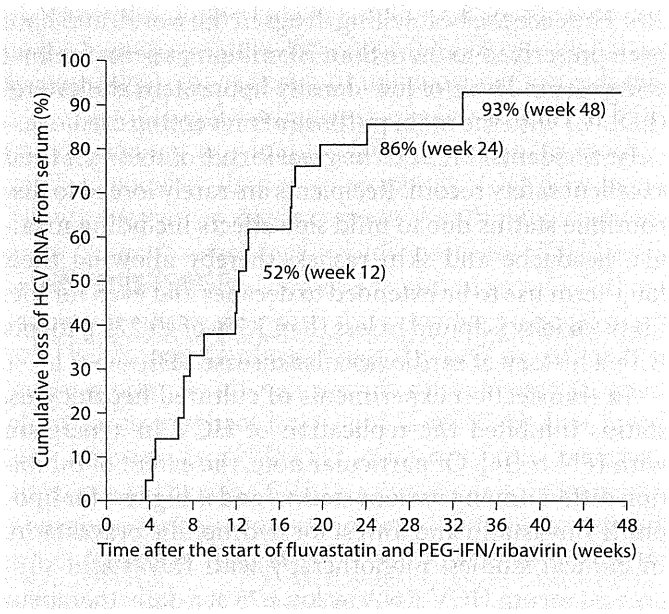

Fig. 1. Kaplan-Meier table illustrating the cumulative loss of HCV RNA from the serum in patients with chronic hepatitis C. The patients received fluvastatin in addition to PEG-IFN/ribavirin and were followed during 48 weeks of treatment.

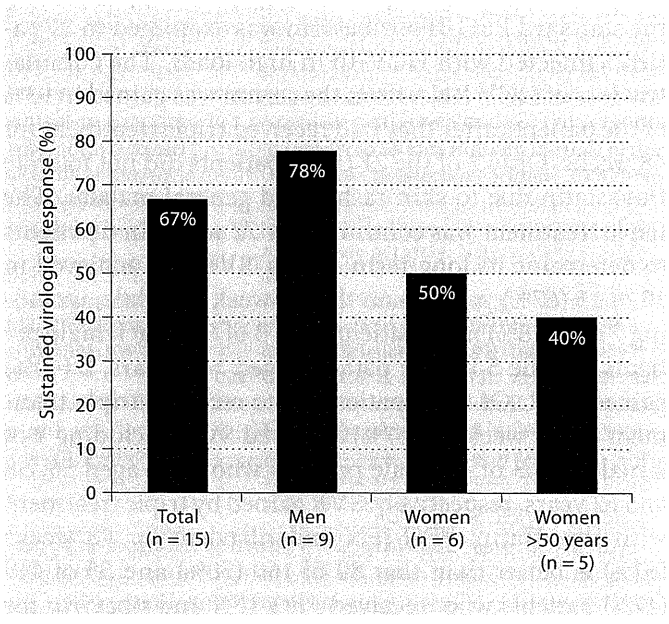

Fig. 2. Sustained virological response in patients with chronic hepatitis C to the combined treatment with fluvastatin, PEG-IFN and ribavirin for 48 weeks. SVR was compared among the total, male and female patients, as well as the female patients aged more than 50 years.

therapy was assessed in the remaining 15 patients. Figure 2 shows the SVR in 15 patients. SVR was accomplished in 10 (67%) of them, including 7 of 9 (78%) male patients and 3 of 6 (50%) female patients. Of the 5 female patients aged >50 years, 2 (40%) gained SVR.

Of the 6 patients who received triple treatment for 72 weeks, SVR was accomplished in 4 (67%), including 1 of 2 male and 3 of 4 female patients who were aged 56, 58 and 62 years, respectively.

Discussion

Statins comprise a group of drugs capable of inhibiting HMG-CoA reductase and can regulate the synthesis of cholesterol by competing with the authentic substrate [19,

20]. They are the best selling drugs in the world, and have been prescribed to more than 30 million patients for lowering serum levels of low-density lipoprotein cholesterol (LDL-C) with the main purpose of preventing cardiovascular accidents [21, 22]. The great virtue of statins is their excellent safety record. Recipients are rarely forced to discontinue statins due to mild side effects including myalgia, headache and skin rashes, thereby allowing their long-term use to be extended to decades and even for life. Liver toxicity is found in less than 1.2% of 49,275 patients with a history of cardiovascular disease [23].

In transfection experiments of cultured hepatocytes, statins inhibited the replication of HCV in synergism with IFN-α [14]. Of particular note, the extent of inhibition differs among various statins, and is highest for lipophilic fluvastatin and lowest for hydrophilic pravastatin. In clinical studies, monotherapy with fluvastatin suppressed serum HCV RNA by log 1.75 at a daily therapeutic dose of <80 mg [13], while no such effects were gained with 20 mg atorvastatin daily [24]. Hence, the ability to inhibit HCV replication may differ among diverse statins through mechanisms not directly associated with lowered levels of LDL-C; fluvastatin decreases total cholesterol and LDL-C levels to a lesser extent than the other statins in current use.

In this open-label pilot study, the response to triple treatment with fluvastatin 20 mg daily supplementing the standard PEG-IFN/ribavirin was examined in 21 patients infected with HCV-1b in high loads. The cumulative loss of HCV RNA from the serum was gained in 93% of the patients after they had received triple treatment for 48 weeks. Only 2 of the 21 (10%) patients did not tolerate fluvastatin due to skin rashes and general malaise. The triple treatment was continued for 72 weeks in 6 patients to determine its long-term effects. SVR was achieved in 10 of 15 (67%) patients on the 48-week schedule, including 7 of 9 (78%) male patients and 3 of 6 (50%) female patients. Of the 5 female patients aged >50 years, 2 (40%) gained SVR. Of the 6 patients who received triple treatment for 72 weeks, 4 (67%) achieved SVR, including 1 of 2 male and 3 of 4 female patients who were aged 56, 58 and 62 years, respectively. SVR gained by triple treatment with fluvastatin, PEG-IFN and ribavirin for 48 weeks (67%) is better than that 80 of 160 (50%) and 55 of 118 (47%) patients who received PEG-IFN and ribavirin for 48 weeks [16, 25]; they all were infected with HCV-1b at high loads.

Sex and age are the two principal host factors influencing the response to IFN-based treatment. Although women respond better to combined IFN and ribavirin than men in large-scale multinational studies in Western countries [8, 26], target patients were rather young with the median age of about 40–41 years. Sex-dependent responsiveness to IFN is reversed with age, however, and the response to PEG-IFN/ribavirin is much poorer in female than male patients aged 50 years or older (22/100 [22%] vs. 50/94 [53%], $p < 0.001$) [10]. In view of the estradiols that can prohibit fibrosis in experimental cirrhosis induced in rats [27], levels of female sex hormones, which are lower in women than men older than 50 years [28], could be responsible for the sex difference in response to IFN of patients with chronic hepatitis C. Supplementation of aged female patients with estradiols may increase the response, but it can accelerate physiological osteoporosis [29, 30]. Since statin therapy does not increase the risk of severe hepatotoxicity in patients with chronic hepatitis C [31], fluvastatin may provide aged women with an advantage in gaining SVR to PEG-IFN/ribavirin. Furthermore, fluvastatin does not augment the side effects of other drugs, such as cyclosporine A and warfarin, because it is metabolized by CYP2C9 in the liver, unlike other statins that are disposed by CYP3A4 as other drugs [32].

Should statins interfere with the replication of HCV, it remains an open question how they do it. At least three mechanisms may be conceived for the decrease in HCV replication in patients receiving statins. HCV circulates in association with LDL-C [33], and on that basis, the LDL-C receptor has been proposed for infection of hepatocytes [34–36]. HCV RNA replicates in association with lipid droplets in hepatocytes [37, 38], and therefore, the synthesis of cholesterol blocked by statins can reduce the basis on which HCV thrives. However, these two scenarios seem to stand at odds with the response to IFN being better in patients with higher LDL-C levels [39, 40]. Statins prohibit the synthesis of mevalonate that is modified into geranylgeranyl (containing 20 carbons) and then farnesyl (15 carbons) [19]. They both prenylate most cellular proteins to make them lipophilic toward associations with membranes expressing their biological activities [41]. As an extension to these, the geranylgeranylation of host proteins, which may be required for the replication of HCV, is implicated in the capacity of statins to downregulate HCV infection [42–44]. For that matter, most pleiotropic effects of statins, including a deceased incidence of dementia and Alzheimer disease [45–47], could be associated with the inhibition of prenylation rather than lowered levels of LDL-C in the serum [48].

This study is not without limitations. The number of patients receiving fluvastatin along with PEG-IFN and ribavirin is small and fails to gain statistical power in any comparison with those given PEG-IFN/ribavirin in previous studies. The ability of other statins in improving the response to PEG-IFN/ribavirin has not been examined to discover the mechanism of how fluvastatin improves the response to antiviral treatments. The promising results obtained in this study hopefully will invite further interest for planning clinical trials with extended numbers of patients and targeting elderly women in particular. Meanwhile, it may be worth looking back in the database to examine the response of patients receiving PEG-IFN/ribavirin for chronic hepatitis C, simultaneously with statins for controlling LDL-C levels, to see if they fared better than those not taking statins routinely.

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