Long-term interferon therapy after radiofrequency ablation is effective in treating patients with HCV-associated hepatocellular carcinoma

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Received: 13 February 2010 / Accepted: 31 August 2010 / Published online: 24 September 2010
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Abstract

Purpose This study investigates the usefulness of long-term interferon (IFN) therapy following radiofrequency ablation (RFA) for HCV-associated hepatocellular carcinoma (HCC).

Methods This is a retrospective observational study. Patients underwent pegylated IFN- α /ribavirin combination therapy for 48 weeks and then were maintained on IFN- α administration on average for 68 weeks (mean total duration 116 weeks). Patients who underwent IFN monotherapy were maintained on IFN administration on average for 78 weeks.

Results There were biases in the background factors between the IFN and non-IFN groups. Therefore, a covariate adjustment was performed using the propensity score. An analysis of 20-matched patients from each group showed the 5-year cumulative survival rate was higher in the IFN group than in the non-IFN group (100 and 76%, respectively), and the 3-year cumulative recurrence rate

was significantly lower in the IFN group than in the non-IFN group (38.0 and 64.2%, respectively). In 14 patients (i.e., IFN responders), the serum alanine aminotransferase (ALT) level remained normalized at 30 IU/mL or lower, regardless of disappearance of serum HCV RNA. In these patients, the cumulative recurrence rate was low, the hazard ratio was 0.158 (95% confidence interval = 0.045–0.561, $P = 0.004$), and the serum albumin level was retained.

Conclusion These results show the importance of maintaining the liver function and suggest that long-term IFN administration after RFA inhibits recurrence and contributes to an improved outcome in patients (in particular, IFN responders) who initially develop HCC.

Keywords Hepatitis C virus · Interferon · Hepatocellular carcinoma · Prevention · Radiofrequency ablation

Introduction

More than 500 million people in the world are infected with hepatitis B and C viruses (HBV and HCV, respectively). Persistent infection with these hepatitis viruses is strongly associated with the development of hepatocellular carcinoma (HCC). The HCC patient is ranked fifth among cancer patients throughout the world and the number of deaths from HCC is ranked at third [1]. Its pathogenesis is being progressively elucidated; in most instances, HCC develops in patients who have a background of HBV- and HCV-induced chronic hepatitis and hepatic cirrhosis [2]. Advances in the early detection of and therapy for HCC have increasingly led to curative treatment. However, HCC is likely to recur and the incidences of intrahepatic metastasis and multicentric recurrences are high and are a

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major problem in improving the treatment outcomes [3, 4]. There have been marked advancements recently in interferon (IFN) therapy for chronic hepatitis C. The incidence of HCC is, according to reports, significantly reduced in patients who achieved a sustained viral response (SVR) with IFN therapy and in patients for whom IFN therapy normalizes liver function. This indicates the importance of IFN therapy as a primary prevention against developing HCC [5–9]. IFN therapy after curative treatment of HCV-associated HCC is reportedly useful as secondary prevention against carcinogenesis [10–14]. There are reports that long-term IFN administration inhibits recurrence [15], but the evaluation of this has not been fully established [16]. In this study, after administering radical radiofrequency ablation (RFA), we performed long-term IFN therapy in patients with the initial development of HCV-associated HCC. We retrospectively investigated the recurrence-inhibitory effect of the treatment.

Patients and methods

Patients

Between April 2001 and March 2008, there were 226 patients who underwent RFA at the Division of Hepatobiliary and Pancreatic Diseases, Hyogo College of Medicine. Of these, 135 patients were negative for the HBs antigen and positive for HCV RNA, with tumor diameter ≤ 3 cm, with three or fewer tumors, and underwent RFA alone for the initial development of HCC. These 135 patients were selected for subjects. There were 71 male and 64 female patients who ranged from 39 to 85 years old (the median age was 68 years). The background liver was clinically and histologically evaluated. HCC was diagnosed using abdominal ultrasonography, dynamic computed tomography (CT), and magnetic resonance imaging (MRI). Markedly enhanced tumors were noted in the early phase of contrast imaging on CT or MRI in all patients and washout was observed in the portal phase or late phase 3 min after injection of the contrast medium. RFA was performed with ultrasonographic guiding using the Cool-tip Radiofrequency Ablation system (Tyco Healthcare Group LP, Burlington, MA). The lesions were evaluated by dynamic CT or MRI 1 and 8 weeks after RFA. The treatment was considered markedly effective when the intensely stained tumors disappeared and a treated area was sufficiently maintained.

IFN therapy was indicated for patients meeting the following conditions: (1) platelet count $\geq 70,000 \mu\text{L}^{-1}$, (2) white blood cell count $\geq 1,500 \mu\text{L}^{-1}$, (3) hemoglobin ≥ 10 g/dL, (4) Child-Pugh classification stage A, and (5) age ≤ 80 years. IFN therapy was performed in 20 patients for whom RFA was markedly effective. Nineteen

of 20 patients requested IFN therapy and met the criteria. One patient with Child-Pugh classification stage B underwent IFN therapy because of the patient's strong desire for this therapy. One hundred and fifteen patients did not undergo IFN therapy. Fifty-one of 115 patients did not meet the criteria. IFN therapy was not performed in 64 patients because of their unwillingness to undergo this therapy (despite meeting the indications for treatment) or because ursodeoxycholic acid or strong neominophagen C were being administered instead of IFN because the patients had complications such as hypertension and diabetes (Table 1).

Since this was a retrospective study, covariate adjustment using the propensity score was performed to adjust for between-group confounding factors [17, 18]. The following ten factors were adopted as matching covariates: age, gender, platelet count, serum alanine aminotransferase (ALT) level, α -fetoprotein (AFP) level, protein induced by vitamin K absence or antagonist-II (PIVKA-II) level, Child-Pugh classification, tumor diameter, number of tumors, and the Japanese tumor, node, and metastasis (TNM) stage [19]. Based on the results of the adjustments, 20 patients with IFN therapy and 20 patients without IFN therapy (i.e., IFN and non-IFN groups, respectively) were assessed. Recurrence of HCC was classified as a local tumor progression or as an ectopic recurrence. A local tumor progression was defined as recurrence in an area previously treated with RFA and an ectopic recurrence was defined as a recurrence outside this area.

IFN therapy

IFN therapy that was initiated 1 month after RFA was markedly effective in treating the initial HCC. In the IFN group, 19 patients had the genotype 1b and a high viral load. In Japan, pegylated IFN- α /ribavirin (PEG-IFN/RBV) combination therapy was approved at the end of 2004, and we have planned to give PEG-IFN/RBV combination therapy since 2005. IFN therapy was administered after requests from individual patients who gave informed consent. Twelve patients underwent PEG-IFN/RBV combination therapy. The therapy regimen consisted of PEG-IFN- α -2b (Peg-Intron, Schering Plough Corporation, Kenilworth, NJ) administered at a dose of 1.5 $\mu\text{g/kg/week}$; PEG-IFN- α -2a (Pegasys, Hoffman La Roche, Nutley, NJ) administered at a dose of 180 $\mu\text{g/week}$; and RBV administered for 48 weeks at a dose based on body weight (600 mg for ≤ 60 kg, 800 mg for 60–80 kg, and 1,000 mg for >80 kg). Two of the 12 patients achieved SVR, which was defined as the persistent negative conversion of serum HCV RNA after the 24th week of IFN therapy. After the combination therapy, nine patients underwent IFN- α (Sumiferon, Dainippon Sumitomo Pharma Co., Ltd.,

Table 1 Demographic and baseline characteristics of the patients

Variable	IFN group	Non-1 FN group	<i>P</i>
Patients (<i>n</i>)	20	115	
Age (range), years	65 (52–76)	67 (39–85)	0.06
Gender (<i>n</i>)			0.04
Male	15	56	
Female	5	59	
White blood count ($\times 10^2 \mu\text{L}^{-1}$)	40 (38, 50)	37 (28, 46)	0.08
Hemoglobin (g/dL)	12.9 (11.7, 13.9)	12.8 (11.3, 13.5)	0.35
Platelet count ($\times 10^4 \mu\text{L}^{-1}$)	9.7 (8.7, 11.9)	9.7 (8.0, 12.2)	0.36
Prothrombin activity (%)	80.9 (79.5, 88.9)	81.9 (77.5, 84.2)	0.80
Total bilirubin (mg/dL)	0.8 (0.6, 1.2)	0.9 (0.5, 1.2)	0.51
Albumin level (g/dL)	3.9 (3.5, 4.1)	3.7 (3.3, 3.9)	0.006
AST (IU/L)	60 (33, 82)	57 (45, 86)	0.42
ALT (IU/L)	50 (37, 83)	53 (39, 72)	0.71
AFP (ng/mL)	11 (6, 41)	27 (6, 79)	0.03
PIVKA-II (mAU/mL)	25 (17, 60)	37 (21, 137)	0.95
Child-Pugh classification (<i>n</i>)			0.41
A	19	96	
B	1	18	
C	0	1	
Tumor size (mm)	21 (17, 24)	20 (19, 29)	0.75
Number of tumors (<i>n</i>)			0.17
Solitary	18	88	
Multiple	2	27	
Japanese TNM stage (<i>n</i>)			0.044
I	6	43	
II	13	45	
III	1	27	
Follow-up period (month)	37 (19, 57)	31 (17, 69)	0.13

Except where indicated, data are expressed as the median (25th, 75th percentile)

Osaka, Japan) monotherapy (300 MU, 3 times/week) for a mean duration of 68 weeks (mean total duration 116 weeks).

Eight patients underwent IFN monotherapy. One patient had the genotype 2a, one patient had the hemoglobin of 11.1 g/dL and six patients had low levels of the platelet count ($<100,000 \mu\text{L}^{-1}$). In accordance with Japan package insert of PEG-IFN- α -2b, we planned to undertake IFN monotherapy in eight patients on enough informed consent. Treatment was initiated with PEG-IFN- α -2a monotherapy (90 $\mu\text{g}/\text{week}$ or 90 $\mu\text{g}/2$ weeks) in five patients, and IFN- α monotherapy (300–600 MU, 3 times/week) was initiated in three patients. The therapies were continued in these patients, except for one patient who ultimately achieved SVR. The mean duration of PEG-IFN- α -2a or IFN- α monotherapy was 78 weeks.

Follow-up procedure

After RFA for HCC, liver function testing and tumor marker measurement (AFP, PIVKA-II) were performed

every month in all patients. Abdominal ultrasonography was performed every 3 months and dynamic CT or MRI was performed every 6 months. Histological examination was performed on suspected hypovascular HCC using fine-needle aspiration biopsy. When a recurrence occurred, the patient was admitted for appropriate therapy.

Statistical analysis

For between-group comparisons, the Mann–Whitney *U* test was used for continuous variables and the Chi-square and Fisher exact tests were used for categorical variables. The survival time and HCC recurrence were evaluated using the Kaplan–Meier method and between-group comparisons were performed using the log-rank test. The influence of IFN administration on recurrence after RFA was investigated using the Cox proportional hazards model. Covariate adjustment was performed using the propensity score, as previously reported [17, 18]. The *C*-statistic (ROCKIT software, Kurt Rossmann Laboratories, University of Chicago, Chicago, IL) [20] was used for the goodness-of-fit

index of patients matched using the propensity score. Analysis was performed using statistical software SPSS ver. 16.0 (SPSS, Inc., Chicago, IL) and SAS ver. 9.13 (SAS Institute, Inc., Cary, NC). A two-sided *P* value of less than 5% was regarded as significant.

Results

The virological and adverse effects of IFN therapy

Two of the 19 patients with the genotype 1b who had a high viral load and one patient with the genotype 2a achieved SVR. HCV RNA did not become negative in 14 patients. HCV RNA disappeared in the remaining three patients, but assessing SVR was difficult since the patients were undergoing IFN therapy. Normalization of the serum ALT level to 30 IU/mL or lower occurred in 11 of the 17

non-SVR patients. Since there were no severe adverse events, IFN therapy was continued in 12 patients who underwent PEG-IFN/RBV combination therapy and in eight patients who underwent IFN monotherapy. IFN therapy was suspended due to recurrence of HCC in two patients who underwent IFN monotherapy, but the treatment was resumed after a second RFA treatment in the patients. In the non-IFN group, normalization of the serum ALT level to 30 IU/mL or lower did not occur in any patient.

Recurrence of HCC

After radical RFA in 135 patients, the 1- and 3-year cumulative recurrence rates were 5.0 and 38.0%, respectively, in the IFN group, and 25.0 and 68.6%, respectively, in the non-IFN group. The lower rate in the IFN group was statistically significant (*P* = 0.007). A comparison of the

Table 2 Demographic and baseline characteristics of the matched patients

Variable	IFN group	Non-IFN group	<i>P</i>
Patients (<i>n</i>)	20	20	
Age (range), years	66 (52–76)	67 (56–73)	0.30
Gender (<i>n</i>)			1.00
Male	15	15	
Female	5	5	
White blood count ($\times 10^2$ μL^{-1})	40 (38, 50)	39 (29, 46)	0.14
Hemoglobin (g/dL)	12.9 (11.7, 13.9)	13.3 (11.9, 14.2)	0.67
Platelet count ($\times 10^4$ μL^{-1})	9.7 (8.7, 11.9)	10.9 (7.8, 14.6)	0.49
Prothrombin activity (%)	80.9 (79.4, 88.9)	83.8 (74.3, 90.1)	0.89
Total bilirubin (mg/dL)	0.8 (0.6, 1.2)	1.0 (0.7, 1.2)	0.30
Albumin level (g/dL)	3.9 (3.5, 4.1)	3.7 (3.3, 4.0)	0.08
AST (IU/L)	60 (33, 82)	62 (41, 87)	0.19
ALT (IU/L)	50 (37, 83)	52 (35, 67)	0.69
AFP (ng/mL)	11 (6, 41)	18 (8, 69)	0.29
PIVKA-II (mAU/mL)	25 (17, 61)	33 (18, 47)	0.75
Child-Pugh classification (<i>n</i>)			1.00
A	19	20	
B	1	0	
Tumor size (mm)	21 (17, 24)	21 (18, 26)	0.67
Number of tumors (<i>n</i>)			1.00
Solitary	18	18	
Multiple	2	2	
Japanese TNM stage (<i>n</i>)			0.46
I	6	9	
II	13	9	
III	1	2	
HCV genotype			1.00
1 b	19	18	
2a	1	2	
Viral load (kIU/mL)	900 (370, 1,600)	850 (270, 1,250)	0.76
Follow-up period (month)	37 (19, 57)	28 (17, 39)	0.24

Except where indicated, data are expressed as the median (25th, 75th percentile)

background factors between the two groups showed that there were fewer female patients and that the serum albumin level was higher in the IFN group. On the other hand, the AFP level was higher and the Japanese TNM stage was advanced in many of the non-IFN group patients (Table 1). The median follow-up period was 37 and 31 months in the IFN and non-IFN groups, respectively.

Matching was performed using the propensity score to adjust for the background factors. The ten factors, as described above, were adopted for the covariates. Twenty patients were selected from each of the IFN and non-IFN groups through this matching. The *C*-statistic was 0.756. There were no significant differences between the matched patient groups in any of the host, tumor, or viral side background factors (Table 2). The 5-year cumulative survival rates were 100 and 76.9% in the IFN and non-IFN groups, respectively. This difference was not significant ($P = 0.075$) although the rate was higher in the IFN group (Fig. 1a).

HCC recurred after RFA in eight patients in the IFN group and in 12 patients in the non-IFN group. The 1- and 3-year cumulative recurrence rates were 5.0 and 38.0%, respectively, in the IFN group, and 22.2 and 64.2%, respectively, in the non-IFN group. The lower rates in the IFN group were statistically significant ($P = 0.019$) (Fig. 1b).

The patients were classified as “IFN responders” or as “Others”. IFN responders consisted of 3 SVR patients and 11 patients in whom the serum ALT level had normalized at 30 IU/mL or lower on IFN therapy (14 patients in total). The “Others” group consisted of 26 patients. The cumulative recurrence rate was analyzed in the groups. The 1- and 3-year cumulative recurrence rates were 0 and 29.3%, respectively, in the IFN responders group and 20.7 and 63.7%, respectively, in the “Others” group. The lower rates in the IFN responders were statistically significant ($P = 0.001$; Fig. 2). The hazards ratio for recurrence in the IFN responders, based on the Cox proportional hazards model, was 0.158 (95% confidence interval = 0.045–0.561, $P = 0.004$). Local tumor progression was noted in three patients from the “Others” group, and in one patient of the IFN responders ($P = 0.45$; Fig. 3a). The cumulative ectopic recurrence rate was significantly lower in the IFN responders than in the “Others” group ($P = 0.008$; Fig. 3b).

Changes in the serum albumin level were also investigated. The difference between the level immediately before RFA and that measured for data analysis were compared between the two groups. The median duration of the measurement period was 21 and 17 months in the IFN responders and “Others” group, respectively. This difference was not statistically significant ($P = 0.08$). The serum albumin level was retained in the IFN responders, but decreased in the “Others” group ($P = 0.001$; Fig. 4).

Fig. 1 **a** Cumulative survival rates after curative RFA treatment of matched patients with HCC. The cumulative rates were higher in the IFN group than in the non-IFN group ($P = 0.075$). **b** Cumulative recurrence rates after RFA treatment of matched patients with HCC. The recurrence rate of the IFN group was significantly lower than that of the non-IFN group ($P = 0.019$)

Discussion

The mechanism of HCV-associated carcinogenesis has been actively investigated, but it has not been fully elucidated [21]. Therapy for HCC has had marked advancements in recent years. However, this has not sufficiently increased the long-term survival rate. The annual recurrence rate of HCC is as high as 10–25%, even after curative treatment [22, 23]. In many patients, the background liver disease is hepatic cirrhosis, which gradually progresses to liver failure. Advances in treatment methods and determining how to inhibit recurrence are important in improving the prognosis of HCC. The usefulness of IFN therapy as a primary prevention of chronic hepatitis C and hepatic cirrhosis is well-recognized in Japan. IFN therapy apparently inhibits carcinogenesis in patients who achieve SVR [5–9]. We previously reported IFN's carcinogenesis-inhibitory effect

Fig. 2 Cumulative recurrence rates according to efficacy of IFN therapy after curative RFA treatment of matched patients with HCC. The rate of cumulative recurrence of HCC in the IFN responder was significantly lower than the “Others” ($P = 0.001$)

on HCV-induced hepatic cirrhosis [5, 6]. Many clinical studies, mainly in Japan, have confirmed this effect [7–9]. In this study, we performed matching using the propensity score since we noted biases in the background factors between the IFN and non-IFN groups. This method is used in many fields as a covariate adjustment method and modifies the dependent variables in observational studies in which randomized allocation is difficult [24, 25]. The C -statistic was 0.756, showing a favorable matching.

In the matched groups, the cumulative survival rate was higher in the IFN group while the cumulative recurrence rate in this group was significantly lower. IFN exhibited an anti-viral effect, in addition to inhibiting liver cancer cell growth, in a basic study [26]. It also has a clinical anti-tumor effect on HCC [27, 28]. Ikeda et al. [10] and Kubo et al. [11] reported the efficacy of IFN- β and IFN- α , respectively, in inhibiting carcinogenesis after the curative treatment of HCC. Shiratori et al. [12] noted that IFN does not inhibit the initial recurrence of HCC, but it does inhibit subsequent recurrences. We performed long-term IFN monotherapy and prolonged IFN therapy by adding IFN monotherapy after PEG-IFN/RBV combination therapy. This suggests that IFN therapy is useful as a secondary prevention of carcinogenesis. However, the number of patients we used was small. Previous basic study has shown that continuous IFN administration induces anti-tumor effects [29]. Kudo et al. [15] also performed maintenance IFN therapy for HCC after RFA. In their study, the anti-tumor and the carcinogenesis-inhibitory effect of IFN therapy inhibited HCC recurrence and improved treatment outcomes.

Fig. 3 Cumulative recurrence rates after RFA treatment of matched patients with HCC. **a** Rates of local tumor progression was lower than that of the “Others”, but the difference was not statistically significant ($P = 0.45$). **b** Rates of ectopic recurrence of the IFN responder was significantly lower than that of the “Others” ($P = 0.002$)

An analysis of the IFN responder patients and the “Others” groups shows that the IFN-induced reduction of the serum ALT level to 30 IU/mL or lower may be important for inhibiting intrahepatic recurrence. Yoshida et al. [7] performed IFN therapy in patients with chronic type C hepatitis and observed a carcinogenesis-inhibitory effect in biological responders. This was similar to the effect in the SVR patients. Wang et al. [30] reported that high-dose and long-term therapy with IFN- α inhibited intrahepatic tumor recurrence and lung metastasis in nude mice after curative resection. Uenishi et al. [31] performed IFN therapy after surgery for HCC and observed a recurrence-inhibitory effect in patients in whom the serum ALT level normalized, regardless of disappearance of serum HCV RNA. We also observed a low ectopic recurrence rate in the IFN responders. Recent studies of meta-analysis

Fig. 4 Effect of IFN therapy after curative RFA treatment of matched patients with HCC on the levels of serum albumin. The bars indicate mean $\pm$ 1 SD. Serum albumin in the IFN responders was significantly better preserved than the “Others” ($P = 0.001$)

have shown that IFN- α treatment could significantly decrease early recurrence, so-called intrahepatic metastasis, and improved 1-year survival of patients with HCC after complete resection or ablation [32, 33]. This study suggests that IFN therapy is effective for the suppression of intrahepatic metastasis of HCC. This effect of IFN might be related with the direct suppression of tumor growth on an already-existing undetectable malignant lesion. However, mechanisms of IFN’s effect on recurrence were very complex so that no single study could explain them fully.

Jeong et al. [14] reported maintenance of the Child-Pugh score in patients in whom IFN therapy achieved SVR after curative treatment of HCV-associated HCC. This study suggested that the improvement in and the maintenance of the serum albumin level (an important index of liver function) contributed to improved treatment outcomes. In other words, the recurrence is inhibited and liver function is improved in the IFN responders after HCC treatment. This indicates that curative treatment can be performed, even if recurrence occurs. The statistical method used to adjust for the background factors indicated that long-term IFN administration for HCC after RFA inhibited recurrence and improved the treatment outcome—particularly in the IFN responders.

There are several problems with IFN therapy. It is not applicable to patients with a poor liver function. Diverse adverse effects appear with the therapy such as thrombocytopenia. These problems need to be considered in reaching a conclusion concerning IFN administration to prevent recurrence after the curative treatment of HCC. A large-scale prospective study is needed that covers the type and dose of IFN, use of concomitant drugs (such as ribavirin), and the duration of IFN administration.

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