Original Article

Efficacy of interferon-beta plus ribavirin combination treatment on the development of hepatocellular carcinoma in Japanese patients with chronic hepatitis C

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Aim: Although there is much evidence of an antitumor effect of pegylated interferon (IFN)-α-based treatment, limited data is available about that of IFN-β-based treatment. Our goal was to evaluate the impact of IFN-β plus ribavirin (RBV) treatment on the suppression of hepatocellular carcinoma (HCC).

Methods: This retrospective, multicenter study consisted of 124 chronic hepatitis C patients who were treated with IFN-β plus RBV treatment, including 61 with advanced fibrosis and five with pretreatment HCC. All participants were followed for a median of 2.8 years (range, 2.2–3.2) after the end of their antiviral treatment. The data of 112 patients who finished the treatment were available for analysis. Cox proportional hazard analyses were performed to determine factors significantly associated with HCC development. Cumulative incidence curves for HCC were plotted using the Kaplan–Meier method and differences between groups were assessed using the log-rank test.

Results: The 2.9% rate of HCC development of patients with sustained virological response (SVR) was significantly lower (P = 0.027) than the 15.9% of non-SVR patients. Interestingly, no significant difference was observed between the rates of HCC development of patients with and without advanced fibrosis (P = 0.733), even though the SVR rate of patients with advanced fibrosis was significantly lower than that of those without advanced fibrosis (P < 0.001).

Stepwise multivariable Cox analysis extracted that only SVR was significantly associated with HCC development (hazard ratio, 0.20; 95% confidence interval, 0.03–0.84, P = 0.027).

Conclusion: SVR was significantly associated with a lower risk of HCC development after IFN-β plus RBV treatment.

Key words: advanced fibrosis, antitumor, beta-interferon, hepatocellular carcinoma

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Conflict of interest: Norihiro Furusyo has received an investigator initiated study research grant from Janssen Pharmaceutical. He has also received support from Mitsubishi Tanabe Pharma, MSD, Chugai, Daiichi Sankyo and Bristol-Myers. The remaining authors have no conflicts of interest.

Author contribution: H. Ikezaki, H. Nomura and N. Furusyo contributed equally to this work; H. Ikezaki, H. Nomura, N. Furusyo and J. Hayashi designed the research; H. Ikezaki, H. Nomura, N. Furusyo, E. Ogawa, E. Kajiwara, K. Takahashi, A. Kawano, T. Maruyama, Y. Tanabe, T. Satoh, M. Nakamuta, K. Kotoh, K. Azuma, K. Dohmen and S. Shimoda performed the research; H. Ikezaki, H. Nomura and N. Furusyo analyzed and interpreted the data; all authors were involved in drafting the paper and approved its final version.

Received 11 May 2015; revision 9 July 2015; accepted 9 July 2015

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INTRODUCTION

Hepatitis C virus (HCV) infection has emerged as one of the most significant causes of chronic liver disease worldwide, and it often leads to cirrhosis and hepatocellular carcinoma (HCC). While treatment for HCV has remarkably improved, HCC is the fifth most common cancer and the second leading cause of cancer-related death worldwide, and its incidence has been increasing over the last 20 years. 

Interferon (IFN)-free treatment using only direct-acting antiviral agents (DAA) has been recently introduced with promising results and is recommended as the current standard-of-care by the American Association for the Study of Liver Disease and the European Association for the Study of the Liver (EASL). However, the natural existence and emergence of DAA-resistant variants that decrease susceptibility to DAA are of potential concern. IFN-free treatment can be 10–20-times more expensive than IFN-based treatment, thus, it may not be affordable in all areas of the world or by all health-care systems. Moreover, the antitumor effect of IFN-free treatment has not as yet been adequately researched. Therefore, it is important to investigate and thoroughly discuss if and when IFN-based treatment, the old standard of care, should be considered for certain patients. Although there is much evidence of an antitumor effect of pegylated (PEG) IFN-α-based treatment, limited data is available about the antitumor effect of IFN-β-based treatment. IFN-β plus ribavirin (RBV) combination treatment has been an approved treatment for chronic hepatitis C by the Japanese Ministry of Health, Labour and Welfare, and some studies have shown that IFN-β plus RBV treatment has equivalent efficacy and milder adverse effects than PEG IFN-α plus RBV combination treatment. We previously reported that IFN-β has a greater antitumor effect than IFN-α, both in vitro and in vivo.

The primary aim of this retrospective, multicenter study was to evaluate the impact of IFN-β plus RBV combination treatment on the suppression of the development of HCC.

METHODS

Patients

The Kyushu University Liver Disease Study (KULDS) Group consists of Kyushu University Hospital and its affiliated hospitals in the Northern Kyushu area of Japan. This prospective study consisted of 129 Japanese patients with chronic hepatitis C aged 18 years or older. The exclusion criteria were: (i) HCC at enrollment or the development of HCC during the treatment period; (ii) histologically diagnosed liver cirrhosis at enrollment; (iii) shortening of treatment due to adverse effects or poor virological response; (iv) extension of the standard treatment duration; (v) positivity for antibody to HIV or hepatitis B surface antigen; (vi) severe depression with suicidal ideation and/or attempt; (vii) clinical or biochemical evidence of hepatic decompensation at entry; (viii) excessive active alcohol consumption (daily intake >40 g of ethanol) or drug abuse; (ix) other forms of liver disease; or (x) treatment with antiviral or immunosuppressive agents prior to enrollment. After exclusions, the data of 112 patients were available for analysis (Fig. 1). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki as updated in 2008 and was approved by the ethics committee of each participating hospital. Informed consent was obtained from all patients before enrollment. The study was registered as a clinical trial on the University Hospital Medical Information Network (ID 000016070).

Figure 1 Flowchart of the study design. Of 129 patients infected with chronic hepatitis C, five were excluded because they had liver cirrhosis. The remaining 124 received interferon-beta plus ribavirin treatment. Of them, 12 discontinued treatment due to adverse effects or insufficient virological response. The data of the remaining 112 patients who completed treatment were available for analysis.
administered p.o. twice a day at a total dose of 600–1000 mg for 24–48 weeks. The initial dose was adjusted according to bodyweight (600 mg for patients weighing ≤60 kg, 800 mg for those between 60 and 80 kg, and 1000 mg for those ≥80 kg). Both IFN-β and RBV were concurrently initiated. The above durations and doses are those approved by the Japanese Ministry of Health, Labour and Welfare." In order to investigate the incidence of HCC after the combination therapy, the length of the follow-up period was calculated from the end of antiviral therapy to the diagnosis of HCC or the last follow-up visit. Serum α-fetoprotein (AFP) and abdominal imaging (ultrasonographic examination or computed tomography) were performed every 3–6 months for each patient. The HCC diagnosis was based on histology or non-invasive criteria according to the EASL guidelines.17

**HCV RNA detection and clinical evaluation**

Blood samples were collected from the patients just before therapy, at weeks 4, 8, 12, 24 and 48 of the therapy, at the end of therapy and at 24 weeks after the last follow-up visit. The serum HCV RNA level at each point was determined by quantitative real-time polymerase chain reaction assay (COBAS TaqMan HCV Test version 2.0; Roche Diagnostics, Basel, Switzerland) with a linear dynamic range of 1.2–8.0 log10 IU/mL.18 Biochemical and hematological tests were performed once each month during therapy. All were measured by standard laboratory techniques in our hospital laboratory. Successful treatment was sustained virological response (SVR), defined as undetectable HCV RNA at 24 weeks after the end of treatment.

**Interleukin-28B polymorphism analysis**

Human genomic DNA was extracted from peripheral blood. Genotyping by the single nucleotide polymorphism of the interleukin 28B (IL-28B) (rs8099917) gene was done using the TaqMan Allelic Discrimination Demonstration Kit (7500 Real-Time PCR system; Applied Biosystems, Foster City, CA, USA). Patients were genotyped as IL-28B TT, TG or GG at the polymorphic site.19

**Assessment of liver fibrosis**

Liver biopsy was performed by experienced hepatologists for 50 (38.8%) of the 129 patients, with the stage of fibrosis (F0–4) and the grade of activity (A0–3) established according to the METAVIR score.20 Antiviral therapy was initiated within 1 month after liver biopsy. Liver cirrhosis was diagnosed for patients with no liver biopsy by: (i) ultrasonographic findings (nodules in the hepatic parenchyma, portal vein >16 mm) (mandatory inspection); (ii) endoscopic findings (varices, portal gastropathy); or (iii) transient elastography (FibroScan value ≥14.9 kPa; the cut-off value that indicates a negative predictive value for cirrhosis is 100%).21 The EASL HCV guidelines of 2011 describe the accuracy of these non-invasive tests of liver fibrosis as sufficient for identifying patients with cirrhosis.22 Moreover, the diagnosis of advanced fibrosis was made based on at least one of the following histological or serological markers at the time of antiviral treatment initiation: (i) stage F3 by liver biopsy; (ii) an aspartate aminotransferase (AST) to platelet ratio index (APRI) of more than 2.0 (calculated by the following formula: \( \text{APRI} = 100 \times \frac{\text{AST level}}{\text{upper limit of normal}} / \text{platelets} [10^9/L] \));23 or (iii) fibrosis-4 (FIB-4) of more than 3.25 (calculated by the following formula: \( \text{FIB}-4 = \frac{\text{age} \times \text{AST} \ [\text{U/L}] \times \sqrt{\text{platelets} [10^9/L]}}{\sqrt{\text{alanine aminotransferase} [\text{U/L}]}} \)).24

**Statistical analysis**

Baseline continuous data are expressed as median (first–third quartiles) and categorical variables are reported as frequencies and percentages. Univariate analyses were performed using the Mann–Whitney U-test to compare continuous variables between groups and the χ²-test for categorical variables. Cox proportional hazards models were carried out to identify variables significantly associated with the development of HCC. First, univariate analysis was performed with all variables. Subsequently, variables of \( P < 0.20 \) in the univariate analysis were entered into a multivariate Cox regression model, followed by stepwise variable selection to achieve the optimal combination of covariates. The results of Cox proportional hazard analyses are expressed as hazard ratios (HR) and their 95% confidence interval (CI). The main outcome of this study was the incidence of HCC. Cumulative incidence curves for HCC were plotted using the Kaplan–Meier method. Differences between groups were assessed using a log–rank test. The time frame for HCC incidence was defined as the time from the end of antiviral therapy to the diagnosis of HCC.

All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA). \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Patient characteristics**

The clinical characteristics of the 112 studied patients are summarized in Table 1. Of them, 68 (60.7%) achieved SVR and nine (8.0%) developed HCC. Age, advanced fibrosis, HCV genotype, levels of AST and serum albumin, platelet count and the APRI value were
significantly associated with treatment outcome. The 2.9% rate for the development of HCC for the SVR patients (2/68) was significantly ($P=0.027$) lower than the 15.9% for the non-SVR patients (7/44). Two (40.0%) of five patients with HCC before treatment developed HCC as did seven (6.5%) of 107 without HCC before treatment ($P=0.051$).

Risk factors for the development of HCC

Of the 112 patients who were followed for a median of 2.8 years (range, 2.3–3.2), nine (8.0%) developed HCC. The data from the Cox proportional hazard analyses are shown in Table 2. By both univariate and multivariate analysis, only SVR was significantly associated with the development of HCC. Interestingly, advanced fibrosis and the APRI and FIB-4 values, commonly reported as risk factors for the development of HCC, were not associated with the development of HCC.

Cumulative incidence of HCC classified by treatment outcome

The Kaplan–Meier curves for the incidence of HCC classified by treatment outcome are shown in Figure 2. A significant difference was found between SVR and non-SVR patients ($P=0.016$, by log-rank test).

### Table 1 Parameters of patients classified by treatment outcome*

| Factors                        | SVR ($n=68$) | Non-SVR ($n=44$) | $P$   |
|--------------------------------|--------------|------------------|-------|
| Men, $n$ (%)                   | 27 (39.7)    | 17 (38.6)        | $>0.999$ |
| Age (years)                    | 60 (49–68)   | 64 (56–71)       | 0.031 |
| IL-28B TT genotype, $n$ (%)    | 55 (80.9)    | 29 (65.9)        | 0.057 |
| Advanced fibrosis, $n$ (%)     | 21 (30.9)    | 32 (72.7)        | $<0.001$ |
| History of pretreatment HCC, $n$ (%) | 2 (2.9)  | 3 (6.8)         | 0.380 |
| HCV genotype 1, $n$ (%)        | 23 (33.8)    | 36 (81.8)        | $<0.001$ |
| Serum HCV RNA level (log IU/mL) | 6.25 (5.56–6.88) | 6.55 (6.00–6.90) | 0.098 |
| Aspartate aminotransferase (U/L) | 42 (28–76)  | 57 (37–102)      | 0.045 |
| Alanine aminotransferase (U/L) | 54 (35–88)   | 59 (33–110)      | 0.463 |
| Serum albumin (g/L)            | 42 (40–45)   | 40 (38–42)       | 0.010 |
| Fasting plasma glucose (mg/dL) | 99 (90–112)  | 102 (89–117)     | 0.765 |
| Fasting insulin (μU/mL)        | 9.0 (6.0–13.3) | 10.0 (7.0–18.0) | 0.089 |
| HOMA-IR                        | 2.1 (1.3–3.7) | 2.8 (1.7–4.3) | 0.212 |
| Total cholesterol (mg/dL)      | 165 (152–201) | 174 (155–195)   | 0.778 |
| Platelet count ($\times 10^{3}$/L) | 176 (126–212) | 117 (82–176) | 0.007 |
| AFP (mg/mL)                    | 4.2 (3.0–6.1) | 7.0 (4.0–13.5)  | 0.287 |
| APRI                           | 0.72 (0.51–1.42) | 1.66 (0.78–3.15) | $<0.001$ |
| FIB-4                          | 2.19 (1.44–3.66) | 4.99 (2.40–7.45) | 0.233 |
| Occurrence of HCC, $n$ (%)     | 2 (2.9)     | 7 (15.9)         | 0.027 |

*Data are shown as median (first–quartile, third–quartile) and number.

AFP, $\alpha$-fetoprotein; APRI, aspartate aminotransferase to platelet ratio index; FIB-4, fibrosis-4; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOMA-IR, Homeostasis Model of Assessment – Insulin Resistance; IL-28B, interleukin-28B; SVR, sustained virological response.

Cumulative incidence of HCC classified by pretreatment HCC status

The Kaplan–Meier curves for the incidence of HCC classified by pretreatment HCC status are shown in Figure 3. A significant difference was found between patients with and without HCC before treatment ($P=0.018$, by log-rank test).

Cumulative incidence of HCC classified by advanced fibrosis status

The Kaplan–Meier curves for the incidence of HCC showed no significant difference between patients with and without advanced fibrosis ($P=0.615$, by log-rank test).

DISCUSSION

W E HEREIN REPORT the results of a retrospective, multicenter and long-term follow-up study performed to evaluate the effect of IFN-β plus RBV treatment on the development of HCC by Japanese patients with chronic hepatitis C. We previously reported that sex, age, platelet count, AFP level and treatment outcome were significant, independent factors for the development of HCC in patients treated with PEG IFN-α-2b plus RBV.7 In this study, both univariate and multivariate Cox proportional
hazard analysis extracted only treatment outcome as significantly associated with the development of HCC. Based on Kaplan–Meier curves, significant differences were found in the development of HCC between the patients who did or did not achieve SVR and between with and without HCC before treatment.

Table 2  Univariate and multivariate Cox proportional hazard analysis for HCC occurrence

| Variables                              | Univariate Stepwise time-dependent multivariate |
|----------------------------------------|-------------------------------------------------|
|                                        | HR (95% CI)                                      | P     |
|                                        | HR (95% CI)                                      | P     |
| Sex (male)                             | 1.19 (0.30–4.52)                                 | 0.792 |
| Age (years)                            | 1.05 (0.99–1.13)                                 | 0.091 |
| IL-28B TT genotype                     | 1.04 (0.25–7.02)                                 | 0.956 |
| Advanced fibrosis                      | 1.40 (0.37–5.65)                                 | 0.616 |
| With HCC pretreatment                  | 5.43 (0.81–22.53)                                | 0.075 |
| HCV genotype 1                         | 3.31 (0.80–22.25)                                | 0.102 |
| Serum HCV RNA level (log IU/mL)        | 1.10 (0.67–2.10)                                 | 0.722 |
| Aspartate aminotransferase (U/L)       | 1.00 (0.99–1.02)                                 | 0.502 |
| Alanine aminotransferase (U/L)         | 1.00 (0.98–1.01)                                 | 0.688 |
| Serum albumin (g/L)                    | 0.24 (0.06–1.28)                                 | 0.092 |
| Fasting plasma glucose (mg/dL)         | 1.00 (0.97–1.01)                                 | 0.860 |
| Fasting insulin (μU/mL)                | 1.02 (0.98–1.04)                                 | 0.340 |
| HOMA-IR                                | 1.01 (0.92–1.06)                                 | 0.702 |
| Total cholesterol (mg/dL)              | 0.98 (0.96–1.02)                                 | 0.248 |
| Platelet count (×10^9/L)               | 1.00 (0.99–1.01)                                 | 0.651 |
| AFP (ng/mL)                            | 1.01 (0.99–1.02)                                 | 0.161 |
| APRI                                    | 1.08 (0.63–1.60)                                 | 0.751 |
| FIB-4                                   | 0.98 (0.82–1.06)                                 | 0.747 |
| SVR                                     | 0.18 (0.03–0.74)                                 | 0.017 |

AFP, α-fetoprotein; APRI, aspartate aminotransferase to platelet ratio index; CI, confidence interval; FIB-4, fibrosis-4; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOMA-IR, Homeostasis Model of Assessment – Insulin Resistance; HR, hazard ratio; IL-28B, interleukin-28B; SVR, sustained virological response.

Figure 2  Cumulative incidence of hepatocellular carcinoma (HCC) after treatment classified by treatment outcome (Sustained virological response (SVR): continuous line, non-SVR: dashed line). The cumulative incidence of HCC was significantly lower for SVR than for non-SVR patients (P = 0.016 by log–rank test).

Figure 3  Cumulative incidence of hepatocellular carcinoma (HCC) after treatment classified by pretreatment HCC status (Without HCC before treatment: continuous line, with HCC before treatment: dashed line). The cumulative incidence of HCC was significantly lower for patients without than for those with HCC before treatment (P = 0.018 by log–rank test).

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Studies have reported that the mortality from any cause or liver transplantation or from any liver-related outcome, was significantly lower for SVR patients than for non-SVR patients and that complete HCV suppression during antiviral treatment may prevent the development of HCC. A number of DAA have recently been developed and regimens without IFN have shown increased SVR rates. As a result of advances in antiviral treatment, almost all patients can experience complete HCV suppression during treatment. It will be necessary to study the impact of virological response on the development of HCC by patients who receive DAA, with and without IFN antiviral treatment.

It is well documented that the recurrence rate of HCC is notoriously high, 20–40% within 1 year and increasing to approximately 80% by year 5, even after an apparently curative treatment. This may be a consequence of insidious spread or metastasis of HCC before curative treatment. Thus, the carcinogenic environment will not be resolved by treatment of HCC. Previous studies suggest that IFN-based treatments may reduce recurrence of HCC. In this study, of five patients with HCC before treatment, two achieved SVR and did not develop HCC. Of the three non-SVR patients, two developed HCC. However, the number of patients with pretreatment HCC was too small to make definitive conclusions.

In addition to liver cirrhosis, advanced fibrosis is a well-established risk factor for the development of HCC. Interestingly, the univariate Cox proportional hazard analysis of this study showed that advanced fibrosis was not associated with the development of HCC. Moreover, other factors relevant to advanced fibrosis or liver cirrhosis, such as APRI, FIB-4, platelet count, serum albumin and AFP were also not associated with the development of HCC. The favorable influence of IFN-β on platelet count may partially explain this result. We have reported that the platelet count increased during IFN-β plus RBV treatment. Previous studies have correlated a lowered platelet count with the development of HCC, therefore, an increase of platelet count may be favorable for preventing the development of HCC. Thus, we believe that IFN-β plus RBV treatment is beneficial for patients with thrombocytopenia who have advanced fibrosis and thus are at risk for the development of HCC.

The limitations of this study include that only 112 patients were analyzed, which reduces the validity of the analysis. Additional studies including a larger number of patients receiving IFN-β plus RBV treatment will be necessary. Second, this study lacks data on obesity, diabetes mellitus and complicated hepatic steatosis, which have been reported to be risk factors for HCV-related HCC. It is known that approximately 40% of chronic hepatitis C patients have hepatic steatosis when alcohol abuse, obesity and diabetes have been excluded. Thus, the influence of hepatic steatosis on the incidence of HCC remains unclear. Third, there is a lack of data on the duration of chronic hepatitis C and cirrhosis status after treatment; thus, we cannot discuss the influence of these factors on the development of HCC or if IFN-β plus RBV treatment affects the progression of cirrhosis after treatment. Finally, all of the patients of this study are Japanese. Therefore, our results may not be generalizable to other ethnic groups.

In conclusion, this study shows that patients who achieved SVR with IFN-β plus RBV treatment had a lower incidence of HCC than those who did not achieve SVR within approximately 3 years after the end of treatment. However, the risk of developing HCC remains, even after HCV eradication; thus, careful screening of patients will continue to be necessary.

ACKNOWLEDGMENTS

We are grateful to Drs Masayuki Murata, Mosaburo Kainuma, Kazuhiro Toyoda, Motohiro Shimizu, Haru Mukae, Takeshi Ibara, Takeo Hayashi, Fujiko Mitumoto-Kaseida, Koji Takayama, Satoshi Hiramine, Rinne Sakemi, Kazuya Ura, Syo Yamasaki, Yoshifumi Kato, Masaru Sakiyama and Yuki Tanaka from the Department of General Internal Medicine, Kyushu University Hospital for assistance with data collection for this study. The authors also thank Mr Yoshitaka Etoh for his excellent lab work on determining IL-28B single nucleotide polymorphism.

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