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□ ORIGINAL ARTICLE □

# Efficacy and Safety of Combination Therapy of Natural Human Interferon β and Ribavirin in Chronic Hepatitis C Patients with Genotype 2 and High Virus Load

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## Abstract

**Objective** The aim of this study was to evaluate the efficacy of combination therapy of natural human interferon-beta and ribavirin in patients infected with hepatitis C virus (HCV) genotype 2 and high virus load.

**Methods** Inclusion criteria were HCV-genotype 2, serum HCV RNA level of ≥100 KIU/mL before combination therapy. A total of 24 were enrolled in this retrospective cohort study. The treatment period of combination therapy was 24 weeks.

**Results** Of the 24 study patients, no patient stopped the treatment due to treatment-related adverse events. The dose of drugs were reduced in 8 patients. Twenty-one of 24 patients (87.5%) had sustained virological response (SVR) by the intention to treat analysis. The rate of negative HCV RNA at 8 week after the initiation of treatment was 18/21 (86%) in patients with SVR and 1/3 (33%) in patients with non-SVR. Logistic regression analysis showed that SVR occurred when serum HCV RNA at 8 week after the initiation of combination therapy was negative (hazard ratio: 40.0; 95% confidence interval=1.75-914.78; p=0.021)

**Conclusion** The combination therapy of IFN-beta and ribavirin offers sufficient safety and efficacy in chronic hepatitis C patients with genotype 2 and high virus load.

**Key words:** chronic hepatitis C, natural interferon-beta, ribavirin, HCV genotype 2

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## Introduction

Current evidence indicates that combination therapy of peginterferon and ribavirin for hepatitis C virus (HCV) is associated with a higher rate of sustained virological response (SVR) compared with interferon (IFN) alone (1-10). SVR in the patients with HCV genotype 2 treated with IFN monotherapy for 24 weeks was about 80% in group of low virus load and about 40-45% in high virus load (11). However, it has been reported that the SVR rate was about 80-90% in patients with genotype 2 and high virus load treated with peginterferon and ribavirin for 24 week (12-14). Hence, IFN-monotherapy has been recommended as a first choice for chronic hepatitis C patients with genotype 2 and low virus-load in Japan. On the other hand, combination therapy of peginterferon and ribavirin has been recommended as a first choice for chronic hepatitis C patients with genotype 2 and high virus-load. Thus, in the present study, we assessed the efficacy of the patients with genotype 2 and high virus load who showed low rate of SVR.

However, the dropout rates in patients treated with combination therapy of peginterferon and ribavirin are higher than those treated with IFN monotherapy (15-17). In particular,

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the adverse events due to combination therapy of IFN and ribavirin have a tendency to occur in elderly patients. Therefore, in the case of elderly patients, the physician in charge often avoids combination therapy of IFN and ribavirin due to side effects. However, recently, the life-span has been long in Japan. Thus, there is an ongoing need to refine treatment strategies with a strong effect and safety in HCV patients.

Festi et al reported that IFN-beta has sufficient tolerability (15). However, IFN-beta monotherapy does not result in a satisfactory outcome in patients with a high virus load (11). Enomoto et al have reported that IFN-beta plus ribavirin therapy might seem to have a strong effect and mild side effects originating from treatment (18, 19). However, to date there is little information regarding IFN-beta plus ribavirin therapy for chronic hepatitis C.

Thus, in the present study, we performed a retrospective study to examine the efficacy of combination therapy of IFN-beta and ribavirin in patients with genotype 2 and high virus load.

## Materials and Methods

### Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 2a or 2b; 2) serum level of HCV RNA of ≥100 KIU/mL before combination therapy; 3) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 4) no hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) detectable in serum, determined by radioimmunoassay; 5) leukocytes >2,000/mm$^3$, platelet count >80,000/mm$^3$, and bilirubin <2.0 mg/mL; 6) follow up for >6 months before treatment. We excluded from the study all of the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The physician in charge explained the purpose and method of the combination therapy as well as the potential adverse reactions and informed consent was obtained from each patient.

From December 2004 to May 2008, 24 HCV patients were enrolled in this retrospective cohort study at the study hospital.

A SVR was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver. 2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy (20).

Next, predictors of SVR in patients with undetectable HCV RNA in serum during treatment were assessed. Finally, SVR rate based on the attainment time of negativity of HCV RNA and continuance of negative HCV RNA during combination therapy were examined.

### Combination therapy of IFN-beta and ribavirin

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital and a signed consent form was obtained each patient. Treatment was provided for 24 weeks. IFN-beta (Feron, Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 2-8 weeks initially, followed by three times a week for 16-22 weeks. Ribavirin (Rebetol, Schering-Plough, Osaka, Japan) were given at the dose described based on body weight. The ribavirin dose was adjusted according to body weight (600 mg for ≤60 kg, 800 mg for >60 kg and ≤80 kg, and 1000 mg for >80 kg). The period of daily administration in IFN-beta treatment was determined by the physician. The patients were divided into three groups based on the difference of period of daily administration of IFN-beta at the initial stage of treatment: a 2-week regimen, 10 patients; a 4-week regimen, 5 patients; and an 8-week regimen, 9 patients.

Blood samples were obtained just before and 6 month after combination therapy. The samples were stored at -80℃ until analyzed. Using these blood samples, HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (21). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (22). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay (20). Clinical evaluation and biochemical and hematological tests were performed at 4 weekly intervals.

### Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test, Fisher' exact test, Kruskal Wallis test, and/or logistic regression analysis. The following variables were evaluated as prognostic factors: sex, age, body mass index, a history of interferon therapy, a HCV RNA level, biochemical factors (AST, ALT, triglyceride, HDL-cholesterol, LDL-cholesterol), platelet count, HCV RNA 4, 8, 12 weeks after the initiation of IFN therapy, continuous negative period of HCV RNA during IFN therapy and period of IFN therapy. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

## Result

### Clinical characteristics of the patients

A total of 24 patients were enrolled in the present study. Table 1 shows the characteristics of the patients who received combination therapy. Clinical profiles were as follows: mean age=55.9 years, male/female=11/13, and median

**Table 1. Clinical Backgrounds before Combination Therapy of Peginterferon and Ribavirin in Chronic Hepatitis C Patients**

| Character | value |
|---|---|
| Patients, n | 24 |
| Sex, male (%) | 11(45.8%) |
| Age (yrs) | 55.9±10.2 |
| BMI | 23.0±2.5 |
| A history of IFN (+) | 12 (50.0%) |
| HCV RNA(KIU/mL) | 870 (43-5000) |
| HCV genotype (2a/2b) | 14/10 |
| AST (IU/L) | 71±51 |
| ALT (IU/L) | 130±122 |
| FPG (mg/dL) | 96±13 |
| Triglyceride (mg/dL) | 111±73 |
| HDL cholesterol (mg/dL) | 52±19 |
| LDL cholesterol (mg/dL) | 117±31 |
| Platelet ($10^4$/$mm^3$) | 16.6±4.5 |
| A regimen of daily administration of IFN-beta* (2-week/4-week/8-week) | 10/5/9 |

Data are number of patients (percentage) or mean±standard deviation.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HCV, hepatitis C virus; IFN, interferon;

*The patients were divided into three groups based on the difference of period of daily administration of IFN-beta at the initial stage of treatment: a 2-week regimen of daily administration of IFN-beta, 10 patients; a 4-week regimen, 5 patients; and an 8-week regimen, 9 patients.

(range) HCV-RNA=870(103-5,000) KIU/mL.

### *Safety and tolerance of IFN*

Of the 24 patients included in this study, none of the patients discontinued combination therapy because of IFN-related adverse events. However, 7 out of 24 patients had dose reduction of interferon and/or ribavirin due to side effects. IFN-beta dose reduction was necessary in one case due to the development of neutropenia. RBV dose reduction was applied in 6 patients, due to anemia.

The leukocyte count was 4,700 ± 1,390/$mm^3$ and the platelet count was 166,000 ± 45,000/$mm^3$ before the initiation of IFN therapy, whereas the values were 3,020 ± 1,05/$mm^3$ and 134,000 ± 39,000/$mm^3$, respectively, two weeks after the initiation of the therapy.

### *Efficacy of treatment*

Out of the 24 patients enrolled in the present study, 21 patients (87.5%) had SVR by the intention-to-treat analysis. Patients aged ≥65 years were five in total. Four out of five patients aged ≥65 years had SVR. Table 2 shows the differences in the clinical background between patients with SVR and those without SVR. The rate of negative HCV RNA at 8 weeks after the initiation of treatment was 18/21(86%) in patients with SVR and 1/3 (33%) in patients with non-SVR. Logistic regression analysis showed that SVR occurred when serum HCV RNA at 8 weeks after the initiation of combination therapy was negative (hazard ratio: 40.0; 95% confidence interval=1.75-914.78; P=0.021). Moreover, the SVR was not significantly different based on the difference of period of daily administration of IFN-beta at the initial stage of treatment.

### *Background of non-SVR cases*

Three patients had negative HCV RNA at the end stage of treatment, but showed reappearance of HCV RNA after

**Table 2. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR**

| | SVR (n=21) | Non-SVR (n=3) | p value† |
|---|---|---|---|
| Age (years old) | 56.1 ± 9.1 | 57.0 ± 8.0 | 0.827 |
| Sex (male/female) | 12/9 | 2/1 | 0.449 |
| BMI | 22.9 ± 2.5 | 22.8 ± 2.6 | 1.000 |
| a history of IFN (+/-) | 11/10 | 1/2 | 0.759 |
| HCV-load (KIU/mL) | 794± 786 | 1545± 1797 | 0.759 |
| AST (IU/L) | 69 ± 47 | 44 ± 12 | 0.540 |
| ALT (IU/L) | 83 ± 39 | 70 ± 55 | 0.359 |
| FPG (mg/dL) | 96 ± 13 | 92 ± 3 | 0.813 |
| Triglyceride (mg/dL) | 112 ± 74 | 107 ± 57 | 0.614 |
| HDL cholesterol (mg/dL) | 51 ± 20 | 65 ± 17 | 0.297 |
| LDL cholesterol (mg/dL) | 113 ± 31 | 126 ± 15 | 0.540 |
| Platelet ($10^4$/$mm^3$) | 16.3 ± 4.7 | 17.7 ± 5.3 | 0.701 |
| HCV RNA (+/-) 4W | 9/12 | 2/1 | 0.576 |
| HCV RNA (+/-) 8W | 3/18 | 2/1 | 0.099, 0.021‡ |
| HCV RNA (+/-) 12W | 0/21 | 0/3 | 1.000 |
| Period of daily administration of IFN* (2-week/4-week/8-week) | 9/4/8 | 1/1/1 | 0.925 |

Data are number of patients (percentage) or mean±standard deviation.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HCV, hepatitis C virus; IFN, interferon;

*IFN-beta was given intravenously at a dose of 6 million units (MU) daily for 2-8 weeks, followed by three times a week for 16-22 weeks. Figure of 2, 4, and 8 represents the (week) of daily administration of IFN-beta at the initial stage.

†Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test, Fisher' exact test, Kruskal wallis test.

‡Logistic regression analysis showed that SVR occurred when serum HCV RNA at 8 week after the initiation of combination therapy was negative (hazard ratio: 40.0; 95% confidence interval =1.75-914.78; p = .021)

the termination of treatment. Clinical backgrounds of these three cases with relapse of HCV RNA after the termination of treatment are shown in Table 3. In case 1 and 2, the attainment time of negativity of serum HCV RNA was 12 weeks after the initiation of treatment. In case 3, the adherence of both drugs of IFN-beta and ribavirin was less than two-third compared to scheduled dose.

## Discussion

We have described the efficacy of combination therapy of IFN-beta and ribavirin in patients infected with HCV genotype 2a or 2b. The present study was limited to small size with genotype 2 and HCV-load of ≥100 KIU/mL and high virus load before combination therapy. SVR in the patients with genotype 2 treated with IFN monotherapy for 24 weeks was about 80% in the group with a low virus load and about 40-45% with high virus load (11). Thus, in the present study, we assessed the efficacy of the patients with genotype 2 and a high virus load who showed low rate of SVR. Moreover, 7 of 24 patients did not have a histological examination of the liver within one year before combination

**Table 3. Clinical Backgrounds of Patients with Non-SVR**

| Case | Age/Sex | genotype | HCV RNA | AST/ALT (IU/L) | response* | Adherence (%) | |
|---|---|---|---|---|---|---|---|
| | | | | | | IFN | RBV |
| 1 | 53/M | 2a | 220 | 51/104 | 12W | 104% | 100% |
| 2 | 67/M | 2b | 5000 | 30/27 | 12W | 82% | 84% |
| 3 | 51/F | 2a | 103 | 50/51 | 4W | 62% | 68% |

Data are number of patients (percentage) or mean±standard deviation.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IFN, interferon; RBV, ribavirin

*Response of HCV RNA means attainment time of negativity of serum HCV RNA after the initiation of combination therapy

therapy. Another limitation is that the present study was not a randomized controlled study.

However, several findings from the present study have direct implications for combination therapy for chronic hepatitis C in the future. First, the present results suggest that drop-out rate due to side effects in combination therapy of IFN-beta and ribavirin is low. In the previous study, we have reported that the drop-out rate due to side effects in combination study of peginterferon and ribavirin was 8.4% in 0.5 year after the initiation of treatment and 14.9% in one year (15). In the present study, none of the patients discontinued combination therapy because of IFN-related adverse events.

Secondly, out of 24 patients given the combination therapy, 21 patients had SVR. This SVR rate is similar to that of the 24-week combination therapy of peginterferon and ribavirin reported previously (11-13).

Third, the patients with genotype 2 have the possibility of non-SVR in a regimen for 24-weeks when the attainment time of negativity of serum HCV RNA is longer than 8 weeks after the initiation of combination therapy. This indicates that patients with delayed undetectable HCV RNA should be treated to continue the negativity of serum HCV RNA for a prolonged period of >24 weeks to obtain a high rate of SVR.

IFN-beta should be given intravenously. The intravenous injection is not convenient for treatment compared to intramuscular or subcutaneous injection. However, IFN-beta-related side effects are mild and few compared to combination therapy of IFN-alpha and ribavirin (18, 19). Moreover, IFN-beta-induced mental disorders are milder than those induced by IFN-alpha (23). Thus, IFN-beta could be given in elderly patients of ≥65 years because of mild side effects (24).

In conclusion, the combination therapy of IFN-beta and ribavirin offers sufficient safety and efficacy in chronic hepatitis C patients with genotype 2 and a high virus load.

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