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□ ORIGINAL ARTICLE □

Efficacy and Safety of Combination Therapy of Natural Human Interferon Beta and Ribavirin in Chronic Hepatitis C Patients with Genotype 1b 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 1b.

Methods Inclusion criteria were HCV-genotype 1b, serum HCV RNA level of ≥100 KIU/ml before the initiation of treatment. A total of 40 patients were enrolled in this retrospective cohort study. The treatment period of combination therapy was 48 weeks. Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR. A p value of <0.05 was considered to indicate a significant difference.

Results Of the 40 study patients, ten had mental disorders before the initiation of combination therapy. One of the patients stopped the treatment due to exacerbation of depression and another patient stopped due to a skin rash. Three patients suspended the therapy due to an insufficient response of positive serum HCV RNA at 24 weeks after the initiation of treatment. Thus, 34 patients completed combination therapy. Fifteen had sustained virological response (SVR). The SVR rate in patients who showed negative HCV RNA 8 weeks after the initiation of combination therapy was 86.7% (13/15). On the other hand, the SVR rate in patients who showed positive HCV RNA at 8 weeks was 8% (2/25) (p<.001). Continuous period of negative serum HCV RNA was 33.1 weeks in SVR groups, and 12.5 weeks in non-SVR groups (p<.001).

Conclusion The combination therapy of IFN-beta and ribavirin is a possible therapy selection for patients with type C hepatitis of genotype 1b and high virus load.

Key words: chronic hepatitis C, natural interferon-beta, ribavirin, HCV genotype 1b

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Introduction

Current evidence indicates that combination therapy of peginterferon and ribavirin (RBV) for hepatitis C virus (HCV) is associated with a higher rate of sustained virological response (SVR) compared with interferon (IFN) alone (1-7). Hence, combination therapy of peginterferon and ribavirin has been widely recommended as a first choice for chronic hepatitis C patients with high virus load.

However, the dropout rates in patients treated with combination therapy of peginterferon and ribavirin was higher than those treated with IFN monotherapy (8, 9). Previous studies indicated that 10-16% of patients treated with peginterferon and ribavirin for 48 weeks discontinued the therapy due to adverse effects (2, 10, 11). Thus, there is an ongoing need to refine treatment strategies with strong effect and safety in HCV patients.

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Festi et al have reported that IFN-beta has sufficient tolerability (12). However, IFN-beta monotherapy does not result in a satisfactory outcome in patients with genotype 1b and high virus load (13, 14). Combination of IFN-beta plus ribavirin therapy has possibility to show the strong effect for HCV and mild side effects originated from treatment. But, there is little information regarding combination therapy of IFN-beta plus ribavirin therapy (15, 16). Thus, in the present study, we performed a retrospective study to examine the efficacy and safety of combination therapy of IFN-bata and ribavirin in patients with genotype 1b.

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 1b; 2) serum level of HCV RNA of ≥100 KIU/ml before treatment; 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. Next, 380 patients who had the same criteria and were treated with peginterferon-alpha-2b+ribavirin for 48 weeks were selected as control. The physician in charge explained the purpose and method of the combination therapy as well as the potential adverse reactions to each patient and informed consent was obtained from each patient. This study was approved by the Human Ethics Review Committee of Toranomon Hospital.

From December 2004 to May 2008, 40 HCV patients were enrolled in this retrospective cohort study at the study hospital. Patients were classified into three groups according to their response to combination therapy: rapid virological response (RVR), defined as undetectable HCV RNA at week 4 of combination therapy; early virological response (EVR), defined as undetectable HCV RNA at week 12 of combination therapy; late virological response (LVR), defined as undetectable HCV RNA at week 13 to 24 of combination therapy; non-virological response (NVR), defined as detectable HCV RNA at week 24 of treatment. 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 (17). A virological response (VR) was defined as HCV RNA negativity during the treatment of combination therapy.

Combination therapy of IFN-beta and ribavirin

Treatment was provided for 48 weeks. IFN-beta (Feron, Toray Induststries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 2-8 weeks, followed by three times a week for 40-46 weeks. Ribavirin (Rebetol, Schering-Plough, Pharmaceutical Co., 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 1,000 mg for >80 kg). The period of continuous administration in IFN-beta treatment was decided by the physician. The first continuous period in IFN-beta treatment was as follows; a 2-week regimen in 21 patients, in a, 8 in a 4-week regime, and 11 in an 8-week regimen.

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, Pleasanton, CA) (18). 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 (19). 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 (17). 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's exact test, and Kruskal-Wallis test. 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 8, 12 week after the initiation of IFN therapy, and continuous negative period of HCV RNA during 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 40 patients treated with IFN-beta +ribavirin 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=51.9 years, male/female=28/12, and HCV-RNA=1,530±992 KIU/mL. As for underlying disease, 10 out of 40 had mental disorder such as depression. Patients were classified into three groups according to the difference of response: RVR (n=2), EVR

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

	Total	Response			
		RVR+EVR*	LVR*	NVR*	P
Patients, n	40	15	12	13	
Sex, male (%)	28 (70%)	11 (73%)	9 (75%)	8 (62%)	.723
Age (yrs)	51.9±10.0	49.4±11.7	51.3±7.7	56.1±9.1	.159
BMI*	21.9±3.1	23.2±3.6	21.2±2.9	21.9±2.3	.278
HCV RNA(KIU/mL)*	1502±992	1099±1001	1293±814	2160±1190	.024
AST (IU/L) *	78.7±50.3	85.7±70.0	72.0±35.3	77.8±39.8	.914
ALT (IU/L) *	124.1±103.3	155.8 ± 112.5	100.7 ± 67.1	101.3±64.0	.699
FPG(mg/dL)*	97.8±17.9	91.3±13.0	94.4±8.3	116.5±12.4	.304
Triglyceride (mg/dL)	101.0±43.3	93.6±27.8	85.3±23.7	121.1±44.6	.094
Platelet(10^4/mm^3)	18.1±5.4	19.5±5.3	17.5±6.5	16.1±4.1	.198
Continuous period of IFN-beta (week) †	2/4/8	21/8/11	4/5/6	8/1/3	9/2/2	.073

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

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; EVR, early virological response; FPG, fasting plasma glucose; HCV, hepatitis C virus; IFN, interferon; LVR, late virological response; NVR, non virological response;

†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 40-46 weeks. Figure of 2, 4, and 8 represents continuous period of IFN-beta at the first stage.

(n=13), LVR (n=12) and NVR (n=13). There were no significant differences in several factors in three groups as shown in Table 1.

The clinical profiles of 380 patients treated with peginterferon +ribavirin were as follows: mean age=53.0 years, male/female=254/126, HCV-RNA=980±862 KIU/mL, AST=72±62 IU/L, ALT=102±93 IU/L, and platelet count=16.6±4.6 (10^4/mm^3). No patient had a mental disorder such as depression before combination therapy.

Safety and tolerance of combination therapy

Figure 1 shows the cumulative dropout rate based on side effect of combination therapy. Patients treated with IFN-beta + ribavirin had a tendency of low occurrence of combination therapy-related side effects compared to the patients treated with peginterferon + ribavirin (p=0.085).

Of the 40 patients treated with IFN-beta + ribavirin included in this study, two discontinued combination therapy because of IFN-related adverse events: one patient each had exacerbation of depression at 32 weeks after the initiation of treatment and another patient had aggravation of skin rash at 24 weeks.

Further, 15 of the remaining 38 patients had dose reduction of IFN-beta and/or ribavirin because of side effects. IFN-beta dose reduction was necessary in one case due to the development of neutropenia. RBV dose reduction was applied in 14 patients, due to anemia (n=13) and extensive skin eruption (n=1). In 3 patients the therapy was suspended the therapy because of positive serum HCV RNA at 24-32 weeks after the initiation of treatment.

Figure 2 shows the change of platelet count in combination therapy. The platelet count in patients treated with IFN-beta + ribavirin increased with statistical significance at 4, 12, 24, and 48 weeks after the initiation of combination therapy. On the other hand, the platelet count in patients treated with peginterferon + ribavirin decreased with statistical significance at 2, 4, 12, 24, and 48 weeks after the initiation of combination therapy.

Efficacy of treatment

Out of 40 patients treated with IFN-beta + ribavirin enrolled in the present study, 15 patients (37.5%) had SVR by the intention-to-treat analysis. Table 2 shows the differences in the clinical background between patients with SVR and those without SVR. The SVR rate in patients who showed negative HCV RNA 12 weeks after the initiation of combination therapy was 86.7% (13/15). On the other hand, the SVR rate in patients who showed positive HCV RNA 12 weeks was 8% (2/25) (p<.001). Continuous period of negative serum HCV RNA was 33.1 weeks in SVR groups, and 12.5 weeks in non-SVR groups (p<.001). The SVR was significantly associated with the attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA during combination therapy.

Table 3 shows the SVR based on response and continuous period of negative HCV RNA in patients with VR. A stepwise increase in SVR was observed when patients had response of RVR or EVR (p<0.001, Cochran-Armitage Trend Test). However, this trend was not statistically significant on the continuous period of negative HCV RNA (p=.110) in 27

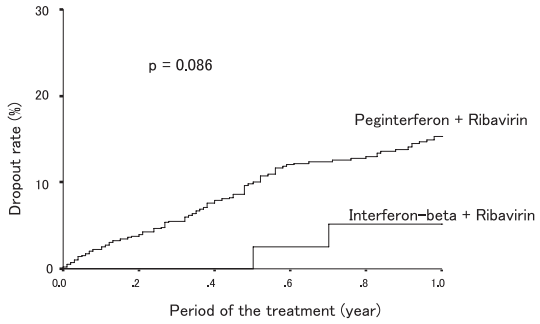

Figure 1. **The cumulative dropout rate based on side effect of combination therapy. Patients treated with IFN-beta + ribavirin had a tendency of low occurrence of combination therapy-related side effects compared to the patients treated with peginterferon + ribavirin (p=0.085).**

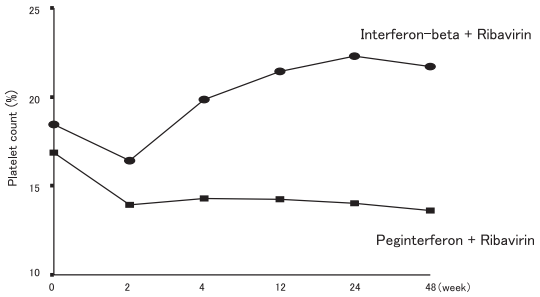

Figure 2. **The change of platelet count in combination therapy. The platelet count in patients treated with IFN-beta + ribavirin increased with statistical significance at 4, 12, 24, and 48 weeks after the initiation of combination therapy. On the other hand, the platelet count in patients treated with peginterferon + ribavirin decreased with statistical significance at 2, 4, 12, 24, and 48 weeks after the initiation of combination therapy.**

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

	SVR * (N=15)	Non-SVR * (N=25)	p value
Age (years old) †	48.2 ± 11.4	54.5 ± 8.3	0.346
Sex (male/female) †	11/4	18/8	0.364
BMI *	23.5 ± 3.0	22.2 ± 2.9	0.710
a history of IFN* (+/-)	10/5	18/7	0.783
HCV-load (KIU/mL) *	1417 ± 1077	1597 ± 958	0.511
AST (IU/L) *	83 ± 67	76 ± 38	0.687
ALT (IU/L) *	150 ± 147	109 ± 65	0.580
FPG(mg/dL)*	91.2± 10.4	100.2± 12.4	0.474
Triglyceride (mg/dL)	81.2± 30.4	113± 45.2	0.422
HDL cholesterol **(mg/dL)**	55.0± 20.9	43.9± 17.1	0.598
LDL cholesterol **(mg/dL)**	121.5± 18.9	122.3± 20.5	0.899
Platelet(10^4/mm^3)	18.9 ± 4.9	16.8 ± 5.8	0.298
HCV RNA (-) 4W	2/15 (13%)	0/25 (0%)	0.133
HCV RNA (-) 12W†	13/15 (87%)	2/25 (8%)	<0.001
HCV RNA (-) 24W	15/15 (100%)	12/25 (48%)	0.001
Continuous negative period (week)	33.1±16.0	12.5 ±22.4	<0.001
Continuous period of IFN-beta (week)‡ 2/4/8	6/5/4	15/3/7	0.125

Data are number of patients, median (range) or mean±standard deviation. P value calculated by the Mann-Whitney U test or Kruskal-Walis test

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

† Serum hepatitis C virus RNA at the 4, 12 and 24 weeks after the initiation of combination therapy

‡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 40-46 weeks. Figure of 2, 4, and 8 represents continuous period of IFN-beta at the first stage.

patients with VR.

Table 4 shows SVR based on adherence of peginterferon and ribavirin. Patients with adherence of >80% in both total dose of IFN-beta and RBV had SVR of 53% (9/17). On ribavirin, a stepwise increase in SVR was observed when the dose of ribavirin increased (p=0.025, Cochran-Armitage Trend Test). However, this trend was not statistically significant for the dose of peginterferon (p=.090).

Discussion

We have described the efficacy of combination therapy of

Table 3. SVR Based on the Attainment Time of Negative HCV RNA and Continuance Period of Negative HCV RNA in Patients with VR during Combination Therapy

Response*	Continuance period of negative HCV RNA (week)				Total
	<20	20-29	30-39	40-49	
RVR	ND	100% (1/1)	ND	100% (1/1)	100%[a] (2/2)
EVR	100% (2/2)	ND	80% (8/10)	100% (1/1)	85%[a] (11/13)
LVR	0% (0/4)	33% (1/3)	20% (1/5)	ND	33%[a] (2/12)
Total	33%[b] (2/6)	50%[b] (2/4)	60%[b] (9/15)	100%[b] (2/2)	56% (15/27)

EVR, early virological response; HCV, hepatitis C virus; LVR, late virological response; ND, not done; RVR, rapid virological response

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

[a]$p<0.001$ for comparison of the 3 groups based to the response (Cochran-Armitage Trend Test)

[b]$p = 0.110$ for comparison of the 4 groups based to the difference of period of negative HCV RNA (Cochran-Armitage Trend Test)

Table 4. SVR Rate Based on Adherence of Combination Therapy

Interferon dose (%)	Ribavirin dose (%)			Total
	≤60	61-80	81-100	
≤60	14%(1/7)	0%(0/2)	ND	11%(1/9)[a]
61-80	0%(0/1)	50%(3/6)	50%(1/2)	44%(4/9)[a]
81-100	0%(0/2)	33%(1/3)	53%(9/17)	45%(10/22)[a]
Total	10%(1/10)[b]	36%(4/11)[b]	53%(10/19)[b]	38%(15/40)

[a]$p = 0.090$ for comparison of the 3 groups based to difference of adherence in peginterferon (Cochran-Armitage Trend Test)

[b]$p = 0.025$ for comparison of the 3 groups based to difference of adherence in ribavirin (Cochran-Armitage Trend Test)

IFN-beta and ribavirin in patients infected with HCV genotype 1b. The present study was limited to patients with genotype 1 and HCV-load of ≥100 KIU/ml. Moreover, in 12 of 40 patients histological examination of the liver was not undertaken within one year before combination 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 drop-out rate due to side effects in combination therapy of IFN-beta and ribavirin was low. In the previous study, we reported that 68 of 612 patients treated with peginterferon and ribavirin stopped the treatment due to side effects and the drop-out rate was 14.9% in one year (9). Moreover, Katamura et al reported that combination therapy of IFN-beta and ribavirin has sufficient tolerability and efficacy in patients with mental disorders (16). This means that combination therapy of IFN-beta and ribavirin might be safe compared with combination therapy of peginterferon and ribavirin.

Secondly, 15 out of 40 patients had SVR. When patients with genotype 1b and high virus load were treated with IFN-beta monotherapy, it has been reported that the SVR rate shows ranges from 0 to 11% (13, 14). Thus, the present study indicates that the combination therapy of IFN-beta and ribavirin is more effective for chronic hepatitis C with genotype 1b and high virus load compared with IFN-beta monotherapy.

Third, SVR had a tendency to occur in patients with RVR or EVR and/or continuance of negative HCV RNA of ≥30 weeks during treatment. On the other hand, SVR rate in patients with LVR was low in a 48-regimen of combination therapy. Previous studies indicated that the suitable treatment period of combination therapy of peginterferon and ribavirin for chronic hepatitis C should be determined based on the time of attainment of negative HCV RNA in patients with genotype 1b and a high virus load of ≥100 KIU/mL (20, 21). Similarly, the present study suggests that in patients with genotype 1b and high virus load, the period of combination therapy should be determined based on the attainment time of negativity of serum HCV RNA.

In a previous study, we reported that virus clearance in combination therapy of peginterferon and ribavirin is associated with HCV mutations in the core region and interferon

sensitivity determinating region, age, gender, LDL-cholesterol, and platelet count. However, the present study does not show that SVR in combination therapy of IFN-beta + ribavirin is associated with age, gender, LDL-cholesterol, and platelet count. The reason is unclear but the following mechanisms might be considered;; 1) sample size of 40 is too small, 2) IFN-beta might have different intracellular mechanisms compared to IFN-alpha. Moreover, unfortunately, we checked HCV mutations in the core region and interferon sensitivity determinating region in only a few patients. Thus, we could not discuss the relationship between HCV mutation and SVR in the present study.

IFN-beta 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 (8, 9). IFN-beta-induced mental disorders are mild compare to those induced by IFN-alpha (16). Moreover, IFN-beta could be given in elderly patients of $\geq$70 years because of mild side effects (22). The mechanism of the better tolerability of IFN-beta and ribavirin is unclear. However, the following mechanism might be possible; 1) IFN-beta is not recombinant IFN but it is produced from human white blood cells. Thus, IFN-beta has a tendency not to produce some immune complex related to IFN-related side effects. 2) IFN-beta might have different intracellular mechanisms compared to IFN-alpha. Although the receptor of IFN alpha and beta are common, intracellular mechanisms could be different from each other. Moreover, it has been reported that IFN-beta reduces the development of bone fracture in postmenopausal women with osteoporosis and chronic liver disease caused by hepatitis C virus (23). Thus, combination therapy of IFN-beta and ribavirin is one possible method for patients who have HCV-genotype 1 and high virus load.

In conclusion, the combination therapy of IFN-beta and ribavirin is a possible therapy selection for patients with type C hepatitis of genotype 1b and a high virus load.

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