

Table 3. SVR rate based on adherence of combination therapy in patients with **A** negativity and **B** positivity of serum HCV RNA at the time of dose reduction¹

A

Total peginterferon dose, %	Total ribavirin dose, %				Total
	<60	61–80	81–100	101–130	
<60	25 (1/4)	40 (2/5)	75 (2/4)	none	38 (5/13)
61–80	0 (0/2)	50 (2/4)	60 (6/10)	100 (2/2)	56 (10/18)
81–100	33 (2/6)	36 (5/14)	50 (7/14)	82 (9/11)	52 (23/44)
101–130	none	none	67 (6/9)	79 (11/14)	77 (17/22)
Total	30 (3/10)	39 (9/23)	57 (21/37)	81 (22/27)	57 (55/97)

B

Peginterferon dose, %	Ribavirin dose, %				Total
	<60	61–80	81–100	101–130	
<60	0 (0/4)	0 (0/2)	0 (0/2)	none	0 (0/8)
61–80	0 (0/2)	0 (0/7)	0 (0/1)	0 (0/4)	0 (0/14)
81–100	0 (0/2)	0 (0/7)	0 (0/2)	20 (1/5)	6 (1/16)
101–130	none	none	0 (0/7)	25 (1/4)	9 (1/11)
Total	0 (0/8)	0 (0/16)	0 (0/12)	15 (2/13)	4 (2/49)

¹ p = 0.08 for comparison of the 4 peginterferon groups and p < 0.001 for comparison of the 4 ribavirin groups (Cochran-Armitage Trend Test).

therapy in patients with negativity of serum HCV RNA at the time of dose reduction. 55 (57%) of 97 patients with undetectable serum HCV RNA at the time of dose reduction had SVR. A stepwise increase in SVR was observed when the dose of ribavirin was increased (p < 0.001, Cochran-Armitage Trend Test). SVR was 42.8% (27/63) in patients who had negativity of serum HCV RNA at the time of dose reduction and had adherence of <100% in both peginterferon and ribavirin. Relapse rate after termination of combination therapy was 43% (42/97) in patients with negativity of serum HCV RNA at the time of dose reduction.

Table 3B shows the SVR rate based on adherence of combination therapy in patients with positivity of serum HCV RNA at the time of dose reduction. Only 2 (2%) of 49 patients with positivity of serum HCV RNA at the time of dose reduction had SVR.

Reasons for Dose Reduction

Of 146 patients with dose reduction, 40 had dose reduction of peginterferon only. 53 patients had dose reduction of ribavirin and 53 patients had both reduction of

peginterferon and ribavirin. The cause of reduction accounted for the following: anemia 87 (59.6%), leukopenia 39 (26.7%), thrombocytopenia 22 (15.1%) and other reason, such as general fatigue, 46 (31.5%). 55 of 146 patients with dose reduction had two causes for dose reduction.

Discussion

We have described the efficacy in patients with dose reduction after the initiation of combination therapy of peginterferon and ribavirin for chronic hepatitis C. The present study was limited to patients with genotype 1 and HCV load of ≥100 KIU/ml because previous studies have suggested that SVR in patients with genotype 2 or 3 is not adversely affected by dose reduction [5, 6, 17]. Another limitation of the study was that patients were treated for different durations. This heterogeneity makes it slightly difficult to interpret the results of the study.

However, several findings from the present study have direct implications for dose reduction of the combination therapy of chronic hepatitis C in the future. First, unde-

detectable serum HCV RNA at the time of dose reduction and attainment of total ribavirin dose of $\geq 100\%$ enhanced SVR in patients with dose reduction. For now, the gold standard of treatment for chronic hepatitis C is a 48-week regimen of combination therapy. Many studies have suggested that reducing the ribavirin dose within the first 12–20 weeks of treatment in patients with genotype 1 was associated with a decline in SVR [8, 9, 18]. The present study indicated that the treatment for >48 weeks and the total ribavirin dose of $\geq 100\%$ enhanced SVR when patients with a dose reduction showed negativity of serum HCV RNA at the time of dose reduction. Second, most patients with detectable HCV RNA at the time of dose reduction did not have SVR regardless of a peginterferon and ribavirin dose of $\geq 100\%$. In patients with detectable HCV RNA at the time of dose reduction, prolonged combination therapy could not enhance SVR.

Several predictive factors of SVR to combination therapy in patients without dose reduction have been identified, and these include amino acid (aa) substitutions in HCV CR (double wild-type; arginine at aa 70/leucine at aa 91), low-density lipoprotein cholesterol (≥ 86 mg/dl), male gender, γ -glutamyl transpeptidase (<109 IU/l), indocyanine green retention test at 15 min ($<10\%$), and ribavirin dose (≥ 11.0 mg/kg) [19]. The present study indicates that HCV RNA at the time of dose reduction and total ribavirin dose were good indicators for predicting SVR in patients with dose reduction.

Some studies have suggested that SVR is increased when patients receive a higher dose of peginterferon and/or ribavirin according to body weight [1, 20]. However, in the present study, when the patients had detectable HCV RNA at the time of dose reduction, they had a slight chance of achieving a SVR regardless of prolonged combination therapy with a total dose of $\geq 100\%$. Thus, the suitable strategies of combination therapy for chronic hepatitis C patients with dose reduction within the first 20 weeks of treatment are as follows: (1) When the patients show undetectable serum HCV RNA at the time of dose reduction, they should be given combination therapy aimed at SVR; they should be treated with combination therapy of peginterferon and total ribavirin dose of $\geq 100\%$. (2) When they show a detectable serum HCV RNA at the time of dose reduction, they should not be given combination therapy aimed at SVR.

In conclusion, on dose reduction of combination therapy in patients with genotype 1b and high virus load, undetectable serum HCV RNA at the time of dose reduction and attainment of the total ribavirin dose of $\geq 100\%$ enhance SVR.

Acknowledgements

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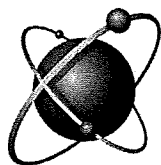
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The Efficacy of Short-Term Interferon-Beta Therapy for Type C Cirrhotic Patients with Genotype 2a and Low Virus Load

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Abstract

Objective The aim of this study was to elucidate the efficacy of short-term interferon (IFN) therapy for type C cirrhotic patients with genotype 2a and low virus load.

Methods The present study was retrospective cohort study. Inclusion criteria were liver cirrhosis, hepatitis C virus (HCV) genotype 2a, the serum HCV RNA level of less than 100 KIU/mL, and IFN period of 6 or 8 weeks. Twenty-five consecutive patients who satisfied the above criteria were treated with IFN-beta daily at the dosage of 6 MU for 6 or 8 weeks. Independent factors that might have influenced sustained virologic response (SVR) were studied using multiple logistic regression analysis.

Results Background of clinical profiles were as follows: median (range) age=64 (53-76) years, male/female=13/12, and median (range) HCV-RNA=31 (8-90) KIU/mL. Out of 25, 14 patients (56.0%) had SVR by the intention-to-treat analysis. The SVR was significantly associated with serum HCV RNA level. Logistic analysis showed that SVR occurred when HCV RNA level was <50 KIU/mL ($p=0.047$). Based on the difference of the serum HCV RNA level, the SVR rate was 68.4% (13/19) in patients with a serum HCV RNA level of <50 KIU/mL and 16.7% (1/6) in patients with a serum HCV RNA level of ≥ 50 KIU/mL.

Conclusions The 6 or 8-week IFN-beta therapy is a possible selection of therapy for cirrhotic patients with HCV genotype 2a and a serum HCV RNA level of <50 KIU/mL.

Key words: liver cirrhosis, hepatitis C virus, genotype 2a, low virus load, interferon, sustained viral response

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Introduction

Current interferon (IFN) therapy for patients with chronic hepatitis C viral infection has been directed at viral clearance. Recent studies have reported improvement of therapeutic efficacy when IFN is combined with ribavirin (1-8). However, IFN is expensive and has a number of serious side effects. Therefore, if the treatment period would become shorter, it could be preferable.

On the other hand, several predictive factors of sustained viral response (SVR) to IFN have been identified, and these

include short duration of disease, young age, absence of liver cirrhosis, genotype 2a, low hepatitis C virus (HCV)-RNA levels, HCV and mutant type of nonstructural5A region (9-15). Patients with liver cirrhosis (LC) have a high development of hepatocellular carcinoma (HCC) and progression to decompensated state. Thus, patients with a cirrhotic state should be treated for protection of progression of LC stage. In particular, LC patients with genotype 2a and low HCV-RNA levels might have the possibility of eradication of HCV RNA with a small dose or a short period of interferon (IFN). However, there is also controversy over how long the IFN therapy should be continued to eradicate HCV RNA in

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Table 1. Clinical Characteristics before Short-term Interferon Therapy in Type C Liver Cirrhosis with Genotype 2a and Low Virus Load

Characteristics	(n=25)
Age (years old)*	64 (53-76)
Male/female [†]	13/12
Period of IFN therapy (6w/8w) [†]	19/6
Total dose of IFN (MU)*	246 (123-336)
HCV load (KIU/mL)*	31 (8-90)
AST (IU/L)*	83 (39-203)
ALT (IU/L)*	74 (27-412)
Hemoglobin (g/dL)*	12.6 (9.7-16.3)
Platelet(10 ⁴ /mm ³)*	11.4 (8.0-17.0)
WBC(10 ³ /mm ³)*	3.8 (3.0-6.9)

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HCV: hepatitis C virus, IFN: interferon, MU: million unit, WBC: white blood cell count.

*Data are expressed as median (range), [†]Data are number of patients.

LC patients with genotype 2a and low HCV-RNA.

Thus, in this study, we evaluated the efficacy of short-term interferon (IFN) therapy for type C cirrhotic patients with genotype 2a and a low virus load.

Materials and Methods

Patients

A total of 25 consecutive cirrhotic type C patients treated with IFN-beta for HCV RNA clearance at Toranomon Hospital in Tokyo, Japan between 2002 and 2006 were enrolled in this study. This study was a retrospective cohort study. Enrollment criteria were: repeated alanine aminotransferase (ALT) elevation of greater than the upper normal limits (ALT normal range: 12-50 IU/L) for more than six months; histological evidence of liver cirrhosis at the time of entry into the trial by the use of distinction equation between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection (16); positive serum HCV RNA; serum HCV RNA level of less than 100 KIU/mL; genotype 2a. We excluded from the study all the patients: 1) with concurrent hepatitis B virus (HBV); 2) with a history of IFN therapy; 3) Leukocytes <3,000/mm³, platelets <80,000/mm³ and bilirubin >1.5 mg/mL before IFN therapy.

Twenty-five patients received IFN at a dose of 6 million units (MU) of natural IFN-beta (Toray Industries or Daiichi Pharmaceutical Co., Tokyo, Japan) daily for 6 or 8 weeks. In general, patients were treated with IFN for 6 weeks and six patients who were treated for 8 weeks were assigned by randomized controlled trial. We regarded sustained virologic response (SVR) to therapy as clearance of HCV RNA by amplicor method (17) for more than 6 months after cessation of therapy. Our study was approved by the institutional ethics review board of our hospital. The physician in charge explained the purpose and method of the clinical trial as well as the potential adverse reactions to each patient, who later gave his/her informed consent for participation.

Blood testing

Blood samples were obtained just before IFN therapy and stored at -80°C. Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems, USA) (18).

On the other hand, serum HCV-RNA at 6 months after the termination of IFN therapy was analyzed by the qualitative PCR assay. The lower detection limit of the qualitative assay is 100 copies/mL. HCV genotype was examined by the PCR assay, using a mixture of primers for the six sub-

Table 2. Predictive Factors for SVR in Short-term Interferon Therapy in Type C Liver Cirrhosis with Genotype 2a and Low Virus Load

Factor	Category	Odds ratio	95% CI	p value*
HCV RNA (KIU/mL)	<50 / ≥50	1/0.09	0.01-0.97	.047
AST (IU/L)	≥76 / <76	1/0.46	0.18-1.17	.102
Age (years)	<60 / ≥60	1/0.22	0.04-1.42	.112
Platelet(10 ⁴ /mm ³)	<10 / ≥10	1/3.00	0.57-15.76	.306
WBC(10 ³ /mm ³)	<4 / ≥4	1/2.33	0.46-11.81	.367
Sex	Male / Female	1/0.71	0.14-3.58	.682
ALT (IU/L)	<100 / ≥100	1/0.75	0.13-4.29	.746
Total dose of IFN (IU/L)	<200 / ≥200	1/1.29	0.23-7.05	.772
Period of IFN therapy (week)	6 / 8	1/1.19	0.20-6.99	.851

ALT: alanine aminotransferase, AST: aspartate aminotransferase, CI: confidence interval, HCV: hepatitis C virus, IFN: interferon, WBC: white blood cell count.

*p value calculated by logistic regression analysis.

types known to exist in Japan, as reported previously (19).

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. Independent factors that might have influenced SVR were studied using multiple logistic regression analysis, and the following variables were evaluated as prognostic factors: sex, age, HCV RNA level, liver histology, biochemical factors (AST (aspartate aminotransferase), ALT) before IFN therapy and methods of IFN administration. 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.

Results

Patients' characteristics

Table 1 shows the characteristics of the 25 patients who

had performed IFN therapy. Clinical profiles were as follows: median (range) age=64 (53-76) years, male/female=13/12, and median (range) HCV-RNA=31 (8-90) KIU/mL. All the patients were categorized as Child-Pugh-Turcotte score class A. Of the 25 patients originally included in this study, in five patients the dose of the IFN therapy was reduced from 6 MU to 3 MU because of general fatigue and thrombocytopenia at the time of 1-3 weeks after the initiation of IFN. Thus, the total dose of IFN was 228.0±79.2 million units (MU). The median (range) leukocyte and platelet count in patients with dose reduction were 3.400 (3.100-4.800)/mm³ and 95.000 (8.8-11.4)/mm³, respectively, while those in patients without dose reduction were 4.600 (3.000-6.900)/mm³ and 120.000 (80.000-120.000)/mm³. Both leukocyte and platelet count in patients without dose reduction were higher than those in patients with dose reduction (leukocyte; p=0.013, platelet; p=0.011).

Efficacy of treatment

Out of twenty-five patients enrolled on present study, 14 patients (56.0%) had SVR by the intention-to-treat analysis.

Table 3. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR

	SVR	Non-SVR	p value
Age (years old) †	7/7	2/9	0.183
(<60/≥60)			
Sex (male/female) †	8/6	5/6	0.647
Period of IFN therapy †	9/5	8/3	0.986
(6 week/8 week)			
Total dose of IFN (MU) † (<200/≥200)	5/9	5/6	0.698
HCV-load (KIU/mL) *	16 (8-69)	66 (23-98)	0.021
AST (IU/L) *	63 (39-203)	85 (53-141)	0.730
ALT (IU/L) *	75 (27-434)	88 (34-230)	0.557
Hemoglobin (g/dL) *	13.7 (10.1-16.3)	11.7 (9.7-16.1)	0.139
Platelet(10 ⁴ /mm ³) *	12.2 (8.7-17.0)	10.0 (8.0-16.0)	0.096
WBC(10 ³ /mm ³) *	4.0 (3.1-6.9)	3.8 (3.0-5.3)	0.841

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HCV: hepatitis C virus, IFN: interferon, MU: million unit, SVR: sustained virologic response, WBC: white blood cell count.

*Data are expressed as median (range), †Data are number of patients,

‡p value calculated by the Mann-Whitney U test.

The SVR was significantly associated with serum HCV RNA level. The patients with a HCV RNA level of <50 KIU/mL tend to have high SVR compared to those with higher than that in patients with HCV RNA level of ≥50 KIU/mL (Table 2). Based on the difference of serum HCV RNA level, the SVR rate was 68.4% (13/19) in patients with a serum HCV RNA level of <50 KIU/mL and 16.7% (1/6) in patients with a serum HCV RNA level of ≥50 KIU/mL. Table 3 shows the differences in the clinical background between patients with SVR and those without SVR. The serum level of HCV RNA in patients with SVR was lower than that in patients without SVR.

Adverse events

Within one week after the initiation of treatment, flu-like symptoms appeared in all the patients. The leukocyte count was 4,320±1,370/mm³ and the platelet count was 119,000±23,000/mm³ before the initiation of IFN therapy, whereas the values were 2,670±830/mm³ and 71,000±17,000/mm³,

respectively, two weeks after the initiation of the therapy. None of the patients withdrew from this treatment due to IFN-related side effects.

Discussion

The present study was limited by non-randomized controlled trial. Another limitation of the study was that the number of the patients was small. However, several findings from the present study have direct implications for the short-term IFN treatment of LC patients with genotype 2a and low virus load.

First, more than 50% of patients cleared HCV RNA. This result indicates that the 6- or 8-week regimen of IFN therapy was preferable to eradicate HCV RNA in LC patients with genotype 2a and low virus load. Second, the patients with HCV RNA level of <50 KIU/mL tend to have high SVR compared to those with higher than that in patients with HCV RNA level of ≥50 KIU/mL. On the treatment

period, the efficacy of the 6-week regimen of IFN therapy was almost the same as that of the 8-week regimen. Moreover, the efficacy of the total dose of IFN of <200 MU was not different from that by the total dose of ≥ 200 MU. These results indicate that in about two-thirds of LC patients with a genotype 2a and serum HCV RNA level of <50 KIU/mL and low virus load, HCV was eradicated by the 6-week regimen or total dose of IFN of <200 MU.

Regarding the side effects of IFN, no patient withdrew the treatment due to IFN-related side effect. Okanoue et al (20) reported that side effects occurred when the daily IFN dose was increased. In the present study, five patients had to reduce the IFN dose due to IFN side effects. On the IFN therapy for LC patients, the physician in charge should check the clinical findings compared to the patients with chronic hepatitis C.

At present, the combined IFN and ribavirin therapy is a standard therapy for chronic hepatitis C patients with genotype 1b and a high load of HCV-RNA. However, prolonged

combination therapy of IFN and ribavirin is associated with various side effects. If the total dose of IFN is decreased and the period of IFN therapy is short, it would be desirable from two points of cost and side effect. Fortunately, in patients with low HCV-RNA levels, HCV RNA tends to be eradicated with a small dose of IFN (21-24). The present study indicates that in patients with a low HCV-RNA, HCV RNA can be eradicated with a small dose of IFN.

Conclusion

The present study indicates that the 6 or 8-week of IFN therapy is a possible selection of therapy for liver cirrhotic type C patients with genotype 2a and low virus load.

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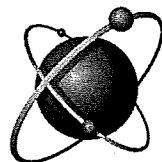
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Suitable Treatment Period in Patients with Virological Response during Combination Therapy of Peginterferon and Ribavirin for Chronic Hepatitis C

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Abstract

Objective The aim of this study was to determine the suitable treatment period in patients who achieve virological response during combination therapy of peginterferon and ribavirin for chronic hepatitis C virus infection.

Methods Inclusion criteria were HCV-genotype 1b, serum HCV RNA level of ≥ 100 KIU/ml before treatment, and negativity of serum HCV RNA during treatment. The 366 patients were enrolled in this retrospective cohort study. Patients were classified into four groups according to difference of response: rapid-virological response (RVR) at week 4 after the initiation of treatment (n=37), early-virological response (EVR) at week 5-12 (n=161), late-virological response (LVR) at week 13-24 (n=131), and superlate-virological response (SLVR) at week 25-48 (n=37). A non-relapse in patients with undetectable HCV RNA during therapy was defined as clearance of HCV RNA 6 month after the cessation of therapy.

Results Of the 366 patients, 241 had non-relapse and the non-relapse rate in each group was 89% (33/37) in RVR, 79% (127/161) in EVR, 54% (71/131) in LVR, and 27% (10/37) in SLVR. In RVR, 26 of 27 patients with continuance of negative HCV RNA of ≥ 30 weeks during treatment had non-relapse. In EVR, patients with period of negative HCV RNA of ≥ 40 weeks had non-relapse rate of 90% (71/79). In LVR and SLVR, all nine patients with continuance of negative HCV RNA of ≥ 60 weeks had non-relapse.

Conclusion A suitable treatment period of combination therapy for chronic hepatitis C should be determined based on the time of attainment of negative HCV RNA.

Key words: chronic hepatitis C, peginterferon, ribavirin, suitable treatment period

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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 (1-7). Hence, combination therapy of peginterferon and ribavirin for HCV has been recommended as a first choice for chronic hepatitis C. During combination therapy, the serum

level of HCV RNA becomes negative in about 70 to 80% of patients with genotype 1 and a high virus load. However, about 20 to 30% of patients with negativity of HCV-RNA during combination therapy relapse after termination of 48 week treatment. Thus, there is an ongoing need to refine treatment strategies because many patients do not respond or relapse after treatment.

Jensen et al reported that a rapid virological response (RVR), defined as undetectable HCV RNA at week 4 of treatment with peginterferon α -2a plus ribavirin, was the

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single best predictor of SVR in genotype 1 patients treated for either 24 or 48 weeks in a randomized, multinational trial (8). This indicates that patients with RVR should be treated for a short course-regimen. In contrast, it may be necessary to treat patients without RVR using a long course-regimen.

The selection of duration of treatment and optimum doses of combination therapy is an area of active investigation (9-15). When the patients with chronic hepatitis C have an undetectable HCV RNA during combination therapy, it is not clear how long the physician should continue the treatment. Thus, in this present study, we performed a retrospective study to examine the relationship between attainment time of negativity of serum HCV RNA after the initiation of combination therapy and the continuance period of negative HCV RNA in patients with undetectable HCV-RNA during treatment.

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, or antimitochondrial antibodies detectable in serum, determined by radioimmunoassay; 5) leukocytes $>2,000/\text{mm}^3$, platelet count $>80,000/\text{mm}^3$, and bilirubin <2.0 mg/ml; 6) negativity of serum HCV RNA within 48 weeks after initiation of combination therapy; 7) duration of treatment of ≥ 8 weeks; 8) the initiation time of treatment was from December 2004 to September 2006. We excluded from the study all the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The institutional ethics review board of our hospital approved our study. The physician in charge explained the purpose and method of this clinical trial, as well as the potential adverse reactions, to each patient, who later gave his/her informed consent for treatment. Informed consent was obtained after confirming the negativity of serum HCV RNA during combination therapy. A total of 366 HCV patients were enrolled in this retrospective cohort study at the study hospital.

Patients were classified into four groups according to the response of combination therapy: RVR, defined as undetectable HCV RNA at week 4 after the initiation of combination therapy; early virological response group (EVR), defined as undetectable HCV RNA at week of 5 to 12 of combination therapy; late virological group (LVR), defined as undetectable HCV RNA at week 13 to 24 of combination therapy; and super late virological group (SLVR), defined as undetectable HCV RNA at week 25 to 48 of combination therapy. A non-relapse in patients with undetectable HCV

RNA during combination therapy 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 month after the cessation of combination therapy (16).

Patients were classified into four groups and evaluated according to the difference of clinical and biochemical backgrounds. Next, predictors of no-relapse in patients with undetectable HCV RNA in serum during treatment were assessed by the multiple logistic regression analysis. Finally, the no-relapse rate based on the attainment time of negativity of HCV RNA and continuance of negative HCV RNA during combination therapy was examined.

Combination therapy of peginterferon and ribavirin

For the treatment regimen, the peginterferon (Peg-intron, Schering-Plough Pharmaceutical Co., Osaka, Japan) and ribavirin (Rebetol, Schering-Plough Pharmaceutical Co.) were given at the dose described based on body weight. At the initiation of combination therapy, patients received peginterferon at a median dose of 1.4 $\mu\text{g}/\text{kg}$ (range, 1.3-1.8 $\mu\text{g}/\text{kg}$) subcutaneously each week and oral ribavirin at a median dose of 11.9 mg/kg (range, 9.5-15.4 mg/kg) daily. The peginterferon dose was adjusted according to body weight (60 μg for ≤ 40 kg, 80 μg for >40 kg and ≤ 60 kg, 100 μg for >60 kg and ≤ 80 kg, 120 μg for >80 kg and ≤ 100 kg, and 150 μg for >100 kg). 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).

A total of 202 patients were treated with 48 week regimen and 113 patients were given combination therapy for > 48 week regimen. Treatment for the remaining 51 patients was discontinued because of treatment-related side effects within 48 weeks after the initiation of combination therapy. On the duration of treatment, 126 patients were given combination therapy based on regimen of clinical trial, 142 patients were treated for 48 weeks of within health insurance, and 98 patients were decided by physician's judgement.

Blood samples were obtained just before and 6 months after combination therapy. The samples were stored at -80°C until analysis. Using these blood samples, the HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (17). 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 (18). Serum alanine aminotransferase (ALT), aspartic 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.

Follow-up protocol

The start of follow-up period was defined as the first day that showed negative HCV RNA after the initiation of com-

bination therapy. Clinical evaluation and biochemical and hematological tests were performed at 4 weekly intervals.

Liver Histology before IFN therapy

Liver biopsy specimens were obtained percutaneously under observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm Tohoku University style, Kakinuma Factory, Tokyo), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The biopsy specimens were scored according to the system of Desmet et al (19). Baseline liver histology of chronic hepatitis prior to IFN therapy was classified according to the extent of fibrosis, into four stages: mild (F1), periportal expansion; moderate (F2), portoportal septa; severe (F3), portocentral linkage or bridging fibrosis; liver cirrhosis (F4). Patients with F1 or F2 were considered as non severe fibrosis. Patients with F3 or F4 were considered as severe fibrosis. In 47 patients, a liver biopsy was not available, because the patients declined to have a biopsy taken.

Statistical analysis

Clinical and biochemical backgrounds before combination therapy among groups based on efficacy of treatment were analyzed using Kruskal Wallis test. We used multivariate analysis (multiple logistic regression analysis) to establish which factors contributed to the non-relapse after combination therapy. Results for each variable were transformed into categorical data consisting of two simple original numbers for multivariate analysis. $p < 0.05$ was considered statistically significant. The variables that we use for multivariate analysis were age, sex, liver histology, body mass index (BMI), HCV-RNA load, continuance period of negative HCV RNA, attainment timing of negative HCV RNA, AST, ALT, hemoglobin, white blood cell, and platelet count. Significance of trends in values was determined with Cochran-Armitage trend test. A $p < 0.05$ was considered statistically significant. The SPSS software package (SPSS 11.0 for windows; SPSS Inc., Chicago, IL, USA) was used for analyses.

Results

Clinical characteristics of the patients

A total of 366 patients were enrolled in the present study. Patients were classified into four groups according to the difference of response: RVR ($n=37$), EVR ($n=161$), LVR ($n=131$), and SLVR ($n=37$). The clinical and laboratory characteristics of the patients at baseline are shown in Table 1. There was a significant difference in the serum level of HCV RNA before treatment among the four groups classified based on the difference of response.

Predictors of non-relapse in patients

Of the 366 patients originally included in this study, 241

had non-relapse and the non-relapse rates in each group were 89% (33/37) in RVR, 79% (127/161) in EVR, 54% (71/122) in LVR, and 27% (10/37) in SLVR. Next, predictors for non-relapse were assessed in the total 366 patients. Univariate analysis showed that the following ten factors significantly affected non-relapse as shown in Table 2A. Multivariate analysis indicated that non-relapse occurred when serum HCV RNA at week 12 was negative ($p=0.003$) and continuance of negative HCV RNA during treatment was ≤ 30 weeks ($p=0.017$) (Table 2B).

Non-relapse based on the attainment time of negativity of serum HCV RNA and continuance period of negative HCV RNA

The non-relapse rate based on the attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA during combination therapy are shown in Table 3. In RVR group, 26 of 27 patients with continuance of negative HCV RNA of ≤ 30 weeks during treatment had non-relapse. In EVR group, patients with period of negative HCV RNA of ≤ 40 weeks during treatment had SVR rate of 90% (71/79). In LVR and SLVR group, all nine patients with continuance of negative HCV RNA of ≤ 60 weeks during treatment had non-relapse.

Discussion

We have described the incidence of non-relapse and treatment period to enhance the non-relapse rate in patients with undetectable HCV RNA during combination therapy of peginterferon and ribavirin for chronic hepatitis C. In the present study, 241 (66%) of 366 patients with undetectable HCV RNA during combination therapy of peginterferon and ribavirin for chronic hepatitis C showed non-relapse. One-third of patients with undetectable HCV RNA during combination therapy showed relapse. On the other hand, 128 with detectable HCV RNA during combination therapy showed detectable HCV RNA after the termination of combination therapy. The present study was limited to patients with genotype 1 and HCV-load of ≥ 100 KIU/ml. Other limitation is that the present study was not a randomized controlled study; the treatment period was varied.

However, several findings from the present study have direct implications for the combination therapy of chronic hepatitis C in the future. First, non-relapse in patients with undetectable HCV RNA during treatment was associated with attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA. Early undetectable HCV RNA and prolonged negativity of serum HCV RNA enhanced the non-relapse rate. Earlier studies have reported higher SVR rates in patients with undetectable HCV RNA at week 4 than in those with detectable HCV RNA (8, 20, 21). The present results coincided closely with these earlier results.

Second, in RVR group, patients with continuance of nega-

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

	Total	Response				p
		RVR	EVR	LVR	SLVR	
Patients, n	366	37	161	131	37	
Sex, male (%) [†]	252 (69%)	28 (76%)	119 (74%)	82 (63%)	23 (62%)	.118
Age (yrs) [‡]	51.6±10.4	51.1±7.0	51.5±10.4	52.0±10.2	52.3±11.7	.399
Weight (kg) [‡]	64.9±11.1	65.1±10.2	64.7±10.4	65.1±11.1	65.2±10.4	.917
BMI [‡]	23.5±2.9	23.3±2.8	23.3±2.8	24.1±2.9	24.0±3.2	.248
HCV RNA(KIU/ml) [§]	1400 (100->5000)	430 (100-1200)	1100 (110->5000)	1750 (130->5000)	1900 (300->5000)	<.001
AST (IU/L) [‡]	66±43	59±62	64±43	66±41	70±52	.419
ALT (IU/L) [‡]	94±66	84±52	98±76	91±59	92±65	.321
WBC(10 ³ /mm ³) [‡]	4.9±2.9	5.0±2.5	5.1±3.7	4.8±2.2	4.6±1.4	.432
Hb (g/dl) [‡]	14.7±1.5	14.7±1.6	14.8±1.7	14.7±1.3	14.8±1.3	.887
Platelet(10 ⁴ /mm ³) [‡]	17.7±8.9	17.8±4.6	18.6±9.2	17.1±7.9	17.9±8.5	.616
Fibrosis staging* F1/F2/F3/F4	151/92/56/8	14/7/2/1	76/41/21/3	45/32/27/4	16/12/6/0	.142
Peginterferon/weight (µg/kg) [‡]	1.4±0.3	1.4±0.3	1.4±0.3	1.3±0.3	1.4±0.3	.916
Ribavirin/weight (mg/kg) [‡]	11.8±1.4	11.8±1.6	11.7±1.4	11.8±1.4	11.8±1.5	.895

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; EVR, early virological response; Hb, hemoglobin; HCV, hepatitis C virus; LVR, late virological response; RVR, rapid virological response; SLVR, super late virological response; WBC, white blood cell;

Normal reference ranges 6-50 IU/L for ALT, 11-38 IU/L for AST,

*Data are number of patients

[†]Data expressed as number of patients (percentage)

[‡]Data expressed as mean±standard deviation

[§]Data expressed as median (range)

tive HCV RNA of ≥ 30 week during treatment had non-relapse of >90%. Next in EVR group, patients with continuance of negative HCV RNA of ≥ 40 weeks during treatment had non-relapse of >85%.

Third, in LVR group, patients with continuance of negative HCV RNA of ≥ 60 weeks during treatment had a high non-relapse rate. The fact that all 9 patients in LVR or SLVR group showed non-relapse after continuance of negative HCV RNA of ≥ 60 week during treatment is important and impressive. This indicates that patients with delayed undetectable HCV RNA should be treated to continue the negativity of serum HCV RNA for a prolonged period to achieve non-relapse. As described above, for 85% of non-relapse, the desirable duration of negativity of serum HCV-RNA may be 30 weeks in RVR, 40 weeks in EVR and 60 weeks in LVR.

It is desirable to expose patients with chronic hepatitis C to the shortest duration of treatment possible to reduce the likelihood of adverse events and minimize costs. Long-term

treatment can be associated with serious side effects and is costly (22). The treatment of combination therapy is expensive; a 24-week treatment course costs approximately 20,000 US \$. Regarding the side effects of treatment, previously unreported side effects were not observed in patients treated for more than one year. However, prolonged combination therapy may cause the serious side effects. Accordingly, careful selection of patients for long-term combination therapy is important.

Conclusions

The results of this study underscore the importance of changing the duration of treatment based on the difference of attainment time of negativity of serum HCV RNA in combination therapy for chronic hepatitis C. To attain a non-relapse rate of >85% in patients with undetectable HCV RNA, continuance of negative HCV RNA during treatment are desirable was 30 weeks in RVR group, 40 weeks in

Table 2. Univariate and Multivariate Analyses Identifying Predictors of Non-Relapse Patients with Genotype 1**Table 2A. Univariate Analyses Identifying Predictors of Non-relapse**

Factor	Category	Odds ratio	95% Confidence interval	p value
HCV RNA week 12*	-/+	1/0.29	0.18-0.47	<.001
HCV RNA week 24*	-/+	1/0.19	0.09-0.39	<.001
Continuance period of negative HCV RNA (week)	<30/≥30	1/4.03	2.34-6.93	<.001
Continuance period of negative HCV RNA (week)	<40/≥40	1/3.38	2.06-5.55	<.001
Age (years)	<50/≥50	1/0.44	0.27-0.72	.001
Continuance period of negative HCV RNA (week)	<50/≥50	1/2.67	1.26-5.65	.010
Continuance period of negative HCV RNA (week)	<60/≥60	1/6.66	1.56-28.46	.011
HCV RNA week 4 [†]	-/+	1/0.24	0.07-0.82	.022
Liver fibrosis	F1,F2/ F3,F4	1/0.56	0.33-0.96	.036
HCV-RNA(KIU/mL)	<1000/≥1000	1/0.62	0.38-1.01	.086
ALT (IU/L)	≤50 / >50	1/1.53	0.42-2.56	.101
Hb (g/dL)	<14/≥14	1/1.64	0.99-2.70	.107
Platelet (x10 ⁴ /mm ³)	<15 / ≥15	1/1.42	0.89-2.26	.147
Sex	Male / Female	1/0.75	0.47-1.21	.244
WBC (x10 ³ /mm ³)	<4 / ≥4	1/1.29	0.78-2.12	.325
BMI	<25 / ≥25	1/0.79	0.47-1.31	.351
AST (IU/L)	≤38 / >38	1/1.08	0.64-1.83	.952

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Hb, hemoglobin; HCV, hepatitis C virus; SVR, sustained virological response; WBC, white blood cell;

[†]HCV RNA at week 12, 24 after the initiation of treatment

Table 2B. Multivariate Analyses Identifying Predictors of Non-Relapse

Factor	Category	Odds ratio	95% Confidence interval	p value
HCV RNA week 12*	- / +	1/0.42	0.24-0.74	.003
Continuance period of negative HCV RNA during treatment (week)	<30 / ≥30	1/2.27	1.16-4.47	.017

HCV=hepatitis C virus,

*HCV RNA at week 12 after the initiation of treatment,

EVR, and 60 weeks in LVR.

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Table 3. SVR Based on the Attainment Time of Negative HCV RNA and Continuance Period of Negative HCV RNA during Combination Therapy*

Response of HCV RNA*	Continuance period of negative HCV RNA (week)						Total
	~19	20~29	30~39	40~49	50~59	≥60	
RVR	33(1/3)	75(3/4)	100(4/4)	96(22/23)	100(3/3)	ND	89(33/37)
EVR	30(3/10)	50(3/6)	76(50/66)	88(51/58)	100(10/10)	91(10/11)	79(127/161)
LVR	0(0/6)	37(10/27)	53(27/51)	60(9/15)	72(18/25)	100(7/7)	54(71/131)
SLVR	0(1/9)	18(2/11)	20(1/5)	40(4/10)	ND	100(2/2)	27(10/37)
Total	18(5/28)	38(18/48)	65(82/126)	81(86/106)	82(31/38)	95(19/20)	66(241/366)

EVR, early virological response; HCV, hepatitis C virus; LVR, late virological response; RVR, rapid virological response; SLVR, super late virological response

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

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aminotransferase, CI: confidence interval, EVR: early virological response, Hb: hemoglobin, HCV: hepatitis C virus, LVR: late virological response, RVR: rapid virological response, SLVR: super-late virological response, WBC: white blood cell

Abbreviations: ALT: alanine aminotransferase, AST: aspartate

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Prolonged-Efficacy of Bisphosphonate in Postmenopausal Women With Osteoporosis and Chronic Liver Disease

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Osteoporosis is present often in postmenopausal women. The aim of this retrospective cohort study is to assess the cumulative appearance incidence and predictive factors for bone fracture in postmenopausal women with osteoporosis and chronic liver disease. The patients were 80 postmenopausal women with osteoporosis and chronic liver disease due to hepatitis virus B or C. These patients were given cyclic etidronate therapy within 3 months after diagnosis of osteoporosis (etidronate-group). Another 400 postmenopausal women with osteoporosis and chronic liver disease were selected as controls (control group). Patients in control group were matched 1:5 with etidronate-group for age. Patients in control group were not given any drugs after diagnosis of postmenopausal osteoporosis. The mean observation period was 8.1 years. Four patients in the etidronate-group and 46 in control group developed bone fracture. The 10th year cumulative appearance rates of bone fracture were 4.9% in etidronate-group and 13.8% in control group. Cox regression model showed that the appearance rate of bone fracture decreased with statistical significance in the following cases: (1) patients < 65 years ($P < 0.001$), (2) patients with serum albumin level of ≥ 3.5 g/dl ($P = 0.003$), and (3) patients treated with etidronate ($P = 0.020$). The cumulative survival rate after bone fracture was 82.2% at the second year, and 57.6% at the fifth year. The present study suggests that a serum albumin level of ≥ 3.5 g/dl and cyclic etidronate treatment reduce the appearance of bone fracture. **J. Med. Virol.** 80:1302–1307, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis; osteoporosis; bisphosphonate; bone fracture

BACKGROUND

Hepatitis C virus (HCV) or hepatitis B virus (HBV) is one of the more common causes of chronic liver disease in

world. Chronic hepatitis C or B is an insidiously progressive form of liver disease that relentlessly but progresses silently to cirrhosis and/or hepatocellular carcinoma (HCC) over a period of 10–30 years [Kiyosawa and Furuta, 1991; Alter et al., 1992; Ikeda et al., 1993; Tsukuma et al., 1993]. Additionally, chronic infection due to hepatitis virus has been associated with a variety of extrahepatic complications such as essential mixed cryoglobulinemia, membranoproliferative glomerulonephritis, autoimmune thyroiditis, and sialadenitis [Johnson et al., 1993; Gumber and Chopra, 1995; Pawlotsky et al., 1995].

Bone disease is one of the major complications of chronic liver disease. Bone fracture rate are increased in chronic liver disease, especially postmenopausal women [Rouillard and Lane, 2001]. Etidronate is an organic compound that inhibits osteoclast mediated bone resorption. Intermittent cyclical etidronate has been shown to be useful for the treatment of osteopenia [Storm et al., 1990; Watts et al., 1990; Miller et al., 1997; Emkey and Ettinger, 2006]. In addition, cyclical etidronate has been reported to be an effective means of increasing bone mineral density in patients with cirrhosis [Shiomi et al., 2002]. Bisphosphonates inhibition hold promise for the treatment of patients with hepatic osteodystrophy. However, there is little information on the yearly cumulative incidence and risk factors on the development rate of bone fracture in

Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

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patients with chronic liver disease during prolonged follow-up.

In Toranomon Hospital (Tokyo, Japan), a large number of patients with HCV or HBV-related hepatitis, were found often with hip fracture or vertebral fracture among elderly patients. With this background, the present retrospective cohort study was initiated to investigate the cumulative incidence and risk factors of bone fracture among postmenopausal women with osteoporosis and chronic liver disease.

MATERIALS AND METHODS

Patients

The number of patients who were diagnosed with chronic HCV or HBV infection between April 1994 and March 2004 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan was 15,400. Out of these, 80 postmenopausal women with osteoporosis and chronic liver disease were treated with cyclical etidronate. These 80 consecutive patients treated with cyclical etidronate were regarded as the etidronate-group. Inclusion criteria except etidronate administration were as follows: (1) 55–75 years; (2) postmenopausal osteoporosis; (3) features of chronic hepatitis or cirrhosis diagnosed by ultrasonography and/or computed tomography; (4) positive for anti-HCV and HCV-RNA or hepatitis B surface antigens (HBsAg); (5) negative for antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay or spot hybridization; (6) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; (7) no underlying systemic disease, such as systemic lupus erythematous, rheumatic arthritis. Diagnosis of osteoporosis was based on bone mineral density (AP spine by dual-energy X-ray absorptiometry) less than 2 SD of young adult mean and/or X-ray evidence of vertical trabecular loss. Patients with either of the following criteria were excluded from the study: (1) malignant tumor, (2) advanced and decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites, and (3) a short follow-up period of 6 months or less. The patients in the etidronate-group received 200 mg of cyclical etidronate orally once a day for 2 weeks, followed by a 10-week period without cyclical etidronate.

In the same period, 1,960 women with osteoporosis and chronic liver disease were not treated with bisphosphonate, steroids or hormone replacement therapy. These patients were followed after exercise and taking calcium-rich foods or drugs for osteoporosis. The 1,215 of these 1,960 patients were considered with seven inclusion criteria and three exclusion criteria described in etidronate group. Four hundred subjects in the control group were selected from these 1,215 patients by matching 1:5 with etidronate-group for age. The differences of the cumulative appearance rate of bone fracture in the etidronate-group and control group were compared. Next, predictive factors for bone fracture in both groups were assessed. The physicians in charge explained the purpose and method of this clinical trial to each patient and/or

the patients' family, who gave their informed consent for participation. This study had been approved by Institutional Review Board of Toranomon hospital.

Viral Markers of HCV and HBV

Diagnosis of HCV infection was based on detection of serum HCV antibody and RNA. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche Molecular Systems, Inc., NJ). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI). The used serum samples were stored -80°C before the time of diagnosis of osteoporosis.

Follow-Up

Patients were followed-up monthly to tri-monthly after the diagnosis of osteoporosis in the Toranomon hospital. Physical examination and biochemical tests were conducted at each examination together with a regular check-up using abdominal ultrasonography and/or computed tomography imaging in each patient. When a patient had any symptoms in relation to bone fracture, the physicians in charge further explored the possibility that patient having bone fracture. Forty-seven patients were lost to follow-up. Because the appearance of bone fracture and death was not identified in these 47 patients, they considered as censored data in statistical analysis [Harrington and Fleming, 1983].

Statistical Analysis

Nonparametric procedures were employed for the analysis of background features of the patients, including the Mann–Whitney *U*-test. The cumulative appearance rate of bone fracture was calculated from the time of diagnosis of osteoporosis by using the Kaplan–Meier method. Differences in the development of bone fracture were tested using the log rank test. Independent factors associated with the incidence rate of bone fracture were analyzed by the Cox proportional hazard model. The following nine variables were analyzed for potential covariates for incidence of bone fracture at the time of diagnosis of osteoporosis at our hospital: age, state of liver disease (chronic hepatitis or liver cirrhosis), platelet count, albumin level, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl transpeptidase (γ -GTP), hepatitis virus, and etidronate therapy. A *P* value of less than 0.05 in two-tailed test was considered significant. Data analysis was performed using the computer program SPSS version 11.0.

RESULTS

Patients' Characteristics

Table I shows the characteristics of the 480 women with postmenopausal osteoporosis and chronic liver disease. There were no significant differences in clinical profiles between the etidronate- and the control group.