Table 3 Factors promoting the response to PEG-IFN and ribavirin in multivariate analysis

Factors	Odds ratio	95% Confidence interval	*P*
Male gender	3.50	1.71–7.17	0.001
$ICG_{15} \leq 13.5\%$	2.09	1.07–4.08	0.031
Ribavirin $\geq$ 11.1 mg/kg per day	2.17	1.11–4.25	0.024
Total PEG-IFN $\geq$ 80%	6.96	2.26–21.4	0.001
PEG-IFN/ribavirin $\geq$ 80%	12.66	2.32–71.4	0.003

more severe and less responsive to IFN in patients infected with HCV genotypes 1 and 4 than in those with HCV genotypes 2, 3 and 6 [18–22]. Likewise, high viral loads are associated with rapid progression of liver disease and poor response to IFN [23–25]. In our study, such viral factors were excluded in comparing the response to PEG-IFN and ribavirin between women and men. All the patients were infected with HCV genotype 1b in high viral loads (>100 kIU/ml).

Age influences the severity of chronic hepatitis C [9, 26], and disease progresses faster and response to antiviral therapy is poorer in older patients [23]. There were significant differences in age between female and male patients in our study. The women were older than the men [mean (range) 57 (30–69) years vs 50 (19–66) years, $P < 0.001$], and the proportion of patients $\geq$60 years was higher in women than in men (39% vs 19%, $P < 0.001$). Hence, the response to PEG-IFN and ribavirin was evaluated in patients aged $\geq$50 years and <50 years separately. There were no differences in the response between female and male patients <50 years, during and at the end of the 48-week treatment, as well as 24 weeks thereafter. However, ETR (55% vs 84%, $P < 0.001$) and SVR (22% vs 53%, $P < 0.001$) were gained significantly less often in women than men who were aged $\geq$50 years.

The influence of gender was observed, also, in patients aged $\geq$60 years and those aged 50–60 years. Hence, women would become less responsive than men to PEG-IFN and ribavirin after they had entered their fifties.

From a therapeutic notion, compliance with treatment can alter the response. Since ribavirin accumulates in erythrocytes and induces hemolysis, it is less tolerated in women who tend to be anemic than men without such an inclination [27]. At the baseline, women had lower levels of hemoglobin and ferritin than men. These would have been responsible for the lower tolerance to PEG-IFN and ribavirin in women than men in our study. In fact, $\geq$80% of the dose of PEG-IFN, ribavirin, or both, was tolerated less frequently in women than men ($P < 0.001$ for each). Even in the patients who had received $\geq$80% of the dose, however, the response to PEG-IFN and ribavirin was gained less frequently in women than in men. Again, the difference was due to a significantly lower response in female patients than in male patients aged $\geq$50 years, while the response was no different between those <50 years of age.

Taken altogether, the poorer response to PEG-IFN and ribavirin in women than in men was attributable to impaired response in the female patients aged $\geq$50 years. Older women with chronic hepatitis C, therefore, would be less responsive to the combined treatment with PEG-IFN and ribavirin currently in use. In support of this view, the response to human lymphoblastoid IFN for 24 weeks is dependent on gender and age [28]. The greatest physiological change precipitated in women by aging is a decreased serum concentration of bioavailable estrogen after they enter the menopause [29]. Estrogen has been shown to have an antifibrotic potential in both experimental and clinical studies. In experimental cirrhosis induced by dimethylnitrosamine in rats, administration of neutralizing antibodies to estradiol and ovariectomy enhanced fibrogenesis in female rats [30]. Hepatocytes have the receptor to estrogen [31], and myofibroblastic transformation in hepatic stellate cells of rats is inhibited in culture supplemented with this hormone [32]. Consequently, hepatic fibrosis progresses faster in menopausal women with chronic hepatitis C, and hormone replacement therapy may be able to prevent it [33]. Furthermore, in women aged $\geq$50 years, the number of estrogen receptor in hepatocytes decreases to one-half of that in those aged <50 years. This would stand in further support of the notion that the antifibrotic effects of decreased estrogen levels in patients aged $\geq$50 years with chronic hepatitis C would produce a lesser response to PEG-IFN and ribavirin.

Favorable effects of female sex hormones on hepatitis have long been suggested. Chronic hepatitis C is mild in menstruating women [34]; its activity is suppressed during pregnancy and enhanced after delivery [35]. The velocity of fibrosis progression is extremely low in young women exposed to HCV through mass-administration of immunoglobulin-D. Only two of 184 (1.2%) and four of 1,018 (0.4%) developed cirrhosis over 24 years and 20 years, respectively, in Irish and German studies [36, 37]. It does need to be pointed out, however, that the majority of women in those studies had not been followed beyond the menopause. There is a possibility that chronic hepatitis C may progress at a faster speed during their next few decades. Continued observations of them would be necessary to evaluate the validity of such an assumption.

Although decreased levels of estrogen can explain the enhanced activity of chronic hepatitis C in older women, as well as their concomitant resistance to PEG-IFN and ribavirin, it does not give an account of the better response in men than women who were aged $\geq$50 years. Feminization represented by gynecomastia is common in men

who have developed cirrhosis, and it can increase even in healthy men with age [38]. Possibly in the background of this phenomenon, circulating levels of free estrogen in men exceed those in women, after they enter their fifties, with margins widening with age [29]. It is tempting to speculate that elevated estrogen levels in men with chronic hepatitis C are responsible for their better response to the combination therapy than women who were aged $\geq$50 years. Whether or not such a speculation would hold would have to be evaluated by a comparison of estrogen levels between older men and women with chronic hepatitis C.

Although osteoporosis is an extrahepatic manifestation of chronic hepatitis C [39], hormone replacement therapy has been withheld for fear of potential hepatotoxicity. There is evidence, however, that oral contraceptives inhibit the progression of fibrosis in women [33]. It may lead to the possibility that the response to antiviral treatment in older women with chronic hepatitis C would be improve by substituting estrogen in them. The merit of hormone replacement therapy for them, of course, would need to be balanced against any harmful effects associated with it.

There are limitations in this study. All the patients were infected with genotype 1b in high viral loads. Hence, the results obtained may or may not be extended to patients with chronic hepatitis C who are infected with HCV of other genotypes in low viral loads. The influence of sex hormones needs to be substantiated by their determination in correlation with SVR. These limitations notwithstanding, the results obtained warrant a special caution in the treatment of women older than 50 years due to their lesser responsiveness to PEG-IFN and ribavirin.

Acknowledgment This study was supported, in part, by grants from the Ministry of Health, Labour and Welfare of Japan.

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Sustained Virological Response Reduces Incidence of Onset of Type 2 Diabetes in Chronic Hepatitis C

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Diabetes is present in patients with chronic hepatitis C virus infection. The aim of this retrospective cohort study was to assess the cumulative development incidence and predictive factors for type 2 diabetes after the termination of interferon therapy in Japanese patients positive for hepatitis C virus (HCV). A total of 2,842 HCV-positive patients treated with interferon (IFN) monotherapy or combination therapy with IFN and ribavirin were enrolled. The mean observation period was 6.4 years. An overnight (12-hour) fasting blood sample or a casual blood sample was taken for routine analyses during follow-up. The primary goal was the onset of type 2 diabetes. Evaluation was performed by using the Kaplan-Meier method and Cox proportional hazard analysis. Of 2,842 HCV patients, 143 patients developed type 2 diabetes. The cumulative development rate of type 2 diabetes was 3.6% at 5 years, 8.0% at 10 years, and 17.0% at 15 years. Multivariate Cox proportional hazard analysis revealed that type 2 diabetes development after the termination of IFN therapy occurred when histological staging was advanced (hazard ratio 3.30; 95% confidence interval [CI] 2.06-5.28; $P < 0.001$), sustained virological response was not achieved (hazard ratio 2.73; 95% CI 1.77-4.20; $P < 0.001$), the patient had pre-diabetes (hazard ratio 2.19; 95% CI 1.43-3.37; $P < 0.001$), and age was ≥50 years (hazard ratio 2.10; 95% CI 1.38-3.18; $P < 0.001$). *Conclusion:* Our results indicate sustained virological response causes a two-thirds reduction in the risk of type 2 diabetes development in HCV-positive patients treated with IFN. (HEPATOLOGY 2009;49:000-000.)

Hepatitis C virus (HCV) is one of the more common causes of chronic liver disease in world. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20% to 50% of cases over a period of 10 to 30 years.[1-3] In addition, HCV is a major risk for hepatocellular carcinoma (HCC).[4-8] Moreover, chronic HCV infection has been associated with a variety of extrahepatic complications such as essential mixed cryoglobulinemia, porphyria cutanea tarda, membranoproliferative glomerulonephritis, autoimmune thyroiditis, sialadenitis, and cardiomyopathy.[9-13] Lately, data supporting a link between type 2 diabetes mellitus (T2DM) and chronic hepatitis C infection have been reported.[14,15]

Although there is growing evidence to support the concept that HCV infection is a risk factor for developing T2DM, there have been a few interventional studies confirming this issue. This issue needs to be confirmed with a long-term follow-up of patients with high risk of developing diabetes. Thus, prospective studies including metabolic evaluations are clearly needed to clarify these issues.

With this background in mind, the cohort study was initiated to investigate the cumulative incidence and risk factors of T2DM after prolonged follow-up in HCV-infected patients treated with interferon (IFN) monotherapy or combination therapy with IFN and ribavirin. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

Patients and Methods

Patients. There were 5,890 patients diagnosed with chronic HCV infection and treated with IFN mono-

Abbreviations: CI, confidence interval; FPG, fasting plasma glucose; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response; T2DM, type 2 diabetes mellitus.

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Received September 25, 2008; accepted October 13, 2008.

Supported in part by grants-in-aid from Okinaka Memorial Institute for Medical Research and the Japanese Ministry of Health, Labor, and Welfare.

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Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.22703

Potential conflict of interest: Nothing to report.

therapy or combination IFN + ribavirin therapy between September 1990 and March 2007 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these, 2,842 patients satisfied the following criteria: (1) no evidence of diabetes mellitus for 3 months after the termination of IFN (plasma glucose concentration <126 mg/dL [6.9 mmol/L] in the fasting state, <200 mg/dL [11.0 mmol/L] in casual state and/or 2 hours after a 75-g oral glucose load); (2) features of chronic hepatitis or cirrhosis diagnosed via laparoscopy and/or liver biopsy before the initiation of IFN therapy; (3) positivity for serum HCV RNA before the initiation of IFN therapy; (4) period of ≤1 year of IFN therapy; (5) negativity for hepatitis B surface antigen (HBsAg), antinuclear antibodies, or antimitochondrial antibodies in serum, as determined via radioimmunoassay or spot hybridization; (6) no evidence of HCC nodules as shown on ultrasonography and/or computed tomography; and (7) no underlying systemic disease, such as systemic lupus erythmatosus or rheumatic arthritis.

Patients who were taking medications known to alter glucose tolerance or had illnesses that could seriously reduce their life expectancy or their ability to participate in the trial were excluded from the study. Patients were classified as having normal glucose or pre-diabetes based on fasting plasma glucose (FPG), casual plasma glucose, or 2-hour plasma glucose. The normal glucose group was regarded as having an FPG of <100 mg/dL, casual plasma glucose of <140 mg/dL, and/or 2-hour plasma glucose of <140 mg/dL. The pre-diabetes group was regarded as having an FPG of 100-125 mg/dL, casual plasma glucose of 140-200 mg/dL, and/or 2-hour plasma glucose of 140-200 mg/dL.[16]

Next, we assessed predictive factors for T2DM in chronic hepatitis C patients treated with IFN. The physicians in charge explained the purpose and method of this clinical trial to each patient and/or the patient's family. Informed consent was obtained from all living patients included in the present cohort study. The study was approved by the Institutional Review Board of our hospital.

Outcome Measures. The primary outcome was T2DM, diagnosed by the use of the 2003 criteria of the American Diabetes Association.[16] These criteria include (1) casual plasma glucose ≥200 mg/dL; (2) FPG ≥126 mg/dL; (3) 2-hour post-glucose (oral glucose tolerance test) ≥200 mg/dL.

Laboratory Investigation. 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, version 2.0; Roche, Tokyo, Japan). Hepatitis B surface antigen was tested via radioimmunoassay (Abbott Laboratories, Detroit, MI). The used serum samples were stored at −80°C at the first consultation. Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA. Height and weight were recorded at baseline, and the body mass index was calculated as weight (in kg)/height (in m^2).

Evaluation of Liver Cirrhosis. Liver status of the 2,842 patients was mainly determined via peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas.[17]

Follow-up. The starting time of follow-up was 3 months after the termination of IFN therapy. After that, patients were followed up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check-up. An overnight (12-hour) fasting blood sample or a casual blood sample was taken for routine analyses. These included aminotransferase activities, total cholesterol, platelet counts, and serum HCV RNA level. Three hundred twenty-four patients were lost to follow-up; because the appearance of T2DM and death was not identified in these patients, they were considered as censored data in the statistical analysis.[18] Moreover, patients retreated with antiviral agents were regarded as withdrawals at the time of starting the retreatment of antiviral agents.

Statistical Analysis. The cumulative appearance rate of T2DM was calculated from 3 months after the termination of IFN treatment to the appearance of T2DM using the Kaplan-Meier method. Differences in the development of T2DM were tested using the log rank test. Independent factors associated with the incidence rate of T2DM were analyzed by the Cox proportional hazard model. The following 11 variables were analyzed for potential covariates for incidence of T2DM at the time of termination of IFN therapy at our hospital: age, sex, state of liver disease (chronic hepatitis or liver cirrhosis), body mass index, glucose level, aspartate aminotransferase level, alanine aminotransferase level, type of IFN, total dose of IFN, efficacy of IFN therapy, hypertension, triglyceride level, and total cholesterol level. A P value of less than 0.05 was considered significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

Results

Patient Characteristics. Table 1 shows the characteristics of the 2,842 HCV-positive patients treated with

Table 1. Patient Characteristics

N	2,842
Sex (male/female)	1,778/1,064
Age (years)	51.8 ± 9.0
Height (cm)	163.8 ± 9.1
Body weight (kg)	62.7 ± 11.7
Body mass index	23.3 ± 3.2
Blood pressure (systolic/diastolic, mm Hg)	128 ± 18/77 ± 12
HCV genotype (1b/2a/2b/other)	744/752/290/56
HCV RNA level (KIU/mL)	593 ± 540
Staging (non-LC/LC)	2,649/193
Blood glucose level (normal/prediabetes)	2,601/241
Fasting plasma glucose (mg/dL)	87 ± 24
Triglyceride (mg/dL)	166 ± 31
Total bilirubin (g/dL)	102 ± 56
AST (IU/L)	74 ± 63
ALT (IU/L)	116 ± 102
IFN monotherapy*/combination therapy†	2,417/425
Efficacy of treatment (SVR/non-SVR)	1,175/1,667
Follow-up period (years)	6.4 ± 5.0

Data are expressed as the number of patients or mean ± standard deviation.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LC, liver cirrhosis; SVR, sustained virological response.

*Outbreak of IFN monotherapy: recombinant IFN-α2a, 304 cases; recombinant IFN-α2b, 235 cases; natural IFN-bα, 1,355 cases; natural IFN-β, 522 cases; total dose of IFN = 598 ± 170 MU.

†Outbreak of combination therapy: recombinant IFN-α2b + ribavirin, 175 cases; total dose of IFN = 537 ± 196 MU; total dose of ribavirin = 182 ± 69 g; pegylated IFN-α2b + ribavirin, 250 cases; total dose of pegylated IFN = 4.28 ± 1.17 mg; total dose of ribavirin = 232 ± 60 g.

IFN monotherapy or combination therapy with IFN and ribavirin. The sustained virological response (SVR) rate was 36.7% (886/2417) in IFN monotherapy and 68% (289/425) in IFN + ribavirin therapy. Thus, the number of patients with SVR was 1,175. The mean period after the termination of antiviral drugs was 6.4 years.

Incidence of T2DM in Patients with HCV. A total of 143 patients (102 men and 41 women) developed T2DM during a mean observation period of 6.4 years. Of these, 26 were SVR and 117 were non-SVR. The cumulative development rate of T2DM was determined to be 3.6% at 5 years, 8.0% at 10 years, and 17.0% at 15 years using the Kaplan-Meier method (Fig. 1). The factors associated with the incidence of T2DM in all 2,842 patients treated with IFN therapy are shown in Table 2.

Multivariate Cox proportional hazard analysis revealed that type 2 diabetes development after the termination of IFN therapy occurred when histological staging was advanced (hazard ratio 3.30; 95% confidence interval [CI] 2.06-5.28; $P < 0.001$), sustained virological response was not achieved (hazard ratio 2.73; 95% CI 1.77-4.20; $P < 0.001$), patient had pre-diabetes (hazard ratio 2.19; 95% CI 1.43-3.37; $P < 0.001$), and age was >50 years (hazard ratio 2.10; 95% CI 1.38-3.18; $P < 0.001$). SVR causes a two-thirds reduction of development of T2DM in patients treated with IFN. In addition to SVR, age ≥50 years, liver cirrhosis, and pre-diabetes contribute to a high risk of developing diabetes. The cumulative development rates of T2DM based on difference of age, efficacy of the IFN therapy, histological diagnosis, and glucose level at the starting time of follow-up are shown in Fig. 2.

Fig. 3 shows the impact of reduction due to SVR on the incidence of T2DM in patients with ≥50 years, liver cirrhosis, or pre-diabetes. When patients with age ≥50 years, liver cirrhosis, and pre-diabetes have SVR after IFN therapy, SVR could statistically reduce the onset of T2DM compared with those without SVR.

Discussion

We have described the development incidence of diabetes after the termination of antiviral therapy in HCV-positive patients treated with IFN therapy in the present study. Diabetes has been reported in less than 0.08% of patients treated with IFN[19,20]; thus, to exclude diabetes originating from IFN-related side effects, patients without diabetes for 3 months after the termination of IFN were enrolled in the present study. The present study indicates that the annual incidence of T2DM for a prolonged follow-up after the termination of IFN therapy among HCV patients is 0.8% to 1.0%. The present study was limited by a retrospective cohort trial. We started the present study in 1991 based on the diabetes mellitus criteria published by Fajans.[21] However, after that, diabetes mellitus criteria were revised. We thus rechecked the diagnosis of T2DM based on the diabetes mellitus criteria of 2003 in patients seen prior to 2003.[16] Because of rechecking the diagnosis of T2DM on the basis of diabetes mellitus criteria in 2003, the present study was regarded as a retrospective cohort study. However, the patients were

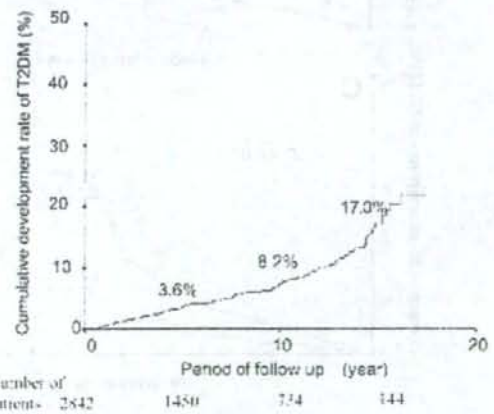

Fig. 1. Cumulative development rate of T2DM in patients treated with IFN.

Table 2. Predictive Factors for T2DM Development

Variables	Univariate Analysis		Cox Regression	
	HR (95% CI)	P Value	HR (95% CI)	P Value
Age, years (≥50/<50)	2.55 (1.74-3.73)	<0.001	2.10 (1.38-3.18)	<0.001
Sex (female/male)	0.84 (0.59-1.19)	0.318		
Body mass index (≥25/<25)	1.44 (0.98-2.08)	0.057		
HCV load (KIU/mL, ≥1,000/<1,000)	0.67 (0.43-1.03)	0.069		
Genotype (1/2)	0.73 (0.50-1.06)	0.098		
ALT (IU/L, ≥50/<50)	1.83 (1.14-2.94)	0.012		
Glucose level (prediabetes/normal)	2.25 (1.53-3.33)	<0.0001	2.19 (1.43-3.37)	<0.001
Triglyceride (mg/dL, ≥150/<150)	1.66 (0.93-2.98)	0.088		
Cholesterol (mg/dL, ≥220/<220)	1.56 (0.62-3.95)	0.346		
Histological diagnosis (LC/non-LC)	4.03 (2.55-6.36)	<0.0001	3.30 (2.06-5.28)	<0.001
Combination of ribavirin (−/+)	1.53 (0.99-2.38)	0.058		
Type of IFN (α/β)	0.88 (0.57-1.35)	0.882		
Total dose of IFN (MU, ≥500/<500)	0.91 (0.59-1.40)	0.672		
Efficacy (non-SVR/SVR)	2.73 (1.77-4.20)	<0.0001	2.78 (1.75-4.41)	<0.001

Data are expressed as the median (range).
Abbreviations: ALT, alanine aminotransferase; HR, hazard ratio; LC, liver cirrhosis.

prospectively followed. Another limitation of the study was that patients were treated with different types of antiviral therapy (IFN monotherapy or combination IFN + ribavirin therapy) for different duration (4 to 52 weeks). This heterogeneity makes it difficult to interpret the results of the study. On the other hand, the strength of the present study is the long-term follow-up in the large numbers of patients included.

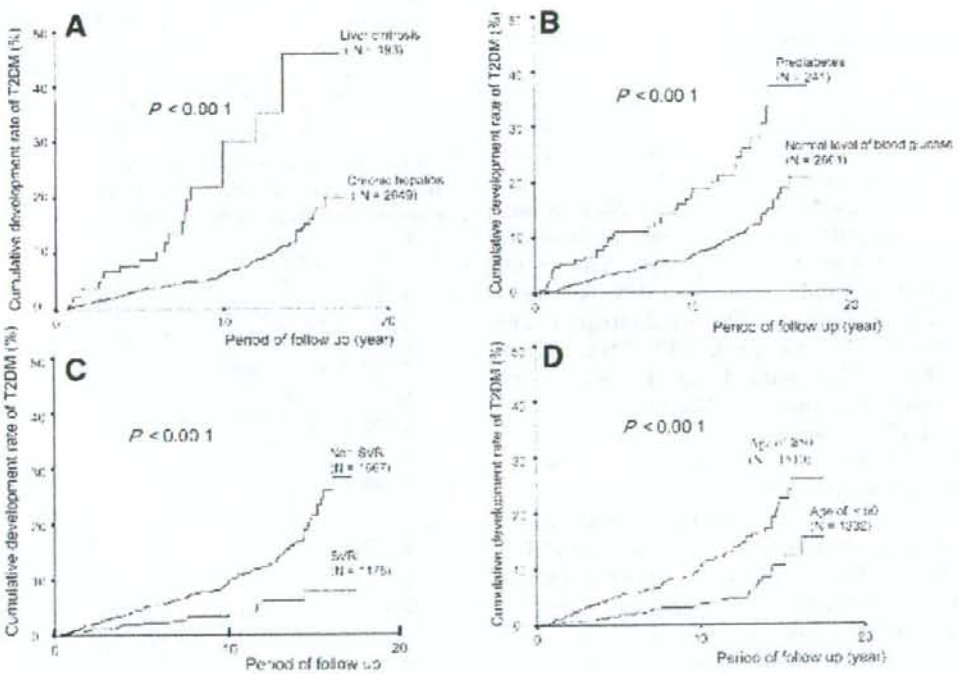

Fig. 2. Cumulative development rate of T2DM in patients treated with IFN. (A) Cumulative development rate of T2DM based on difference of hepatic fibrosis. (B) Cumulative development rate of T2DM based on the difference of glucose level. (C) Cumulative development rate of T2DM based on the difference of efficacy. (D) Cumulative development rate of T2DM based on the difference of age.

Fig. 3. Cumulative development rate of T2DM in patients with SVR or without SVR after IFN therapy. (A) Cumulative development rate of T2DM based on SVR or non-SVR in patients with age ≥50 years. (B) Cumulative development rate of T2DM based on SVR or non-SVR in patients with liver cirrhosis. (C) Cumulative development rate of T2DM based on the difference of SVR or non-SVR in patients with pre-diabetes.

The present study shows several findings with regard to development of T2DM after the termination of antivirus agents for HCV positive patients. First, the T2DM development rate in the non-SVR group was higher than that in the SVR group. The SVR caused a two-thirds reduction in the onset of T2DM in the course of posttreatment follow-up. That SVR reduced the onset of diabetes mellitus in HCV patients is in accordance with the data reported by Simó et al.[22] and Romero-Gómez et al.[23] Though the role of HCV in the pathogenesis of diabetes mellitus remains speculative, the following possible mechanisms have been reported: (1) patients with HCV have a tendency to attain insulin resistance[24]; (2) in transgenic mice, the expression of HCV core protein is associated with insulin resistance and T2DM development[25]; and (3) SVR in HCV patients reduces insulin resistance and onset of the incidence of abnormal glucose value.[26] Thus, it is accepted that clearance of HCV reduces the onset of T2DM.

Second, in addition to persistence of HCV, the present study suggests that aging, histological progression, and pre-diabetes enhanced the onset of T2DM in patients with HCV infection. However, when HCV was eradicated even in patients with age ≥50 years, pre-diabetes, or liver cirrhosis, the cumulative development rate of T2DM decreased.

T2DM is increasing dramatically in many Asian nations, including Japan, over the past decades.[27] It is widely accepted that 7 to 8 million people are affected by diabetes mellitus in Japan. Approximately 8% to 10% of adults in Japan have T2DM. In general, T2DM is associated with a genetic predisposition, but it is also strongly influenced by lifestyle-related factors, such as eating habits and/or physical activity.[28-33] The risk factors associated with T2DM include family history, age, sex, obesity, smoking, and physical activity. T2DM occurred in elderly patients compared to young patients. Life expectancies are long in Japan; thus, in the near future, a large number of patients with HCV will be >60 years of age. Therefore, it is apparent that the incidence of T2DM will increase in HCV-positive patients.

T2DM is a serious, costly disease. Treatment for T2DM may prevent some of its devastating complications, but does not usually restore normoglycemia or eliminate all the adverse consequences.[28,29] Moreover, HCV patients with T2DM are at major risk for HCC.[34] On the efficacy of IFN therapy, it has been reported that T2DM reduces HCV eradication via combination IFN + ribavirin therapy.[26] Thus, it should be considered whether HCV-positive patients should be treated with antiviral drugs in the histological nonprogression stage and at a non-elderly age for prevention of T2DM onset. If SVR obtained via antiviral therapy for HCV cannot only prevent progression to liver cirrhosis or HCC but also prevent the development of diabetes, the potential impact of IFN therapy is quite significant.

In conclusion, this retrospective study suggests that the annual incidence of T2DM among patients with HCV is 0.8% to 1.0%. Our results indicate that SVR causes a two-thirds reduction of T2DM development in HCV-positive patients treated with antiviral drugs.

Acknowledgment: The authors acknowledge the editorial assistance of Thomas Hughes.

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doi:10.1111/j.1440-1746.2008.05319.x

HEPATOLOGY

Association between the treatment length and cumulative dose of pegylated interferon alpha-2b plus ribavirin and their effectiveness as a combination treatment for Japanese chronic hepatitis C patients: Project of the Kyushu University Liver Disease Study Group

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Key words
hepatitis C virus, pegylated interferon alpha-2b, ribavirin.

Accepted for publication 8 November 2007.

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Abstract

Aim: The aim of the present study was to investigate the association between the length of the treatment period and the cumulative dose of pegylated interferon alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) and their effectiveness in the treatment of chronic hepatitis C.

Methods: Seven hundred and fifteen patients received peg-IFN alpha-2b plus RBV treatment for 48 weeks and 24 weeks for genotypes 1 ($n = 586$) and 2 ($n = 129$), respectively.

Results: Sustained virological responses (SVR), defined as serum hepatitis C virus (HCV)-RNA undetectable at 24 weeks after the end of treatment, were 42.4% and 74.4% in genotypes 1 and 2, respectively, on an intention-to-treat analysis. SVR significantly increased with treatment length (4.7%, 36.4%, and 51.8% for < 24 weeks, 24–47 weeks, and 48 weeks, respectively, for genotype 1; and 28.6%, 57.1%, 78.3% for < 12 weeks, 12–23 weeks, and 24 weeks, respectively, for genotype 2). SVR significantly increased with total cumulative treatment dose (21.1%, 36.5%, and 52.9% with < 60%, 60–79%, and ≥ 80% in peg-IFN dose; 29.6%, 51.1%, and 59.2% with < 60%, 60–79%, and ≥ 80% in RBV dose) in genotype 1, although it did not differ significantly for genotype 2.

Conclusions: In peg-IFN alpha-2b plus RBV treatment for chronic hepatitis C, it is important to complete the target length of treatment and to continue the target dosage to achieve SVR, especially for genotype 1 patients.

Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver disease, with perhaps 200 million persons infected worldwide. Approximately 1.8 million patients have chronic HCV infection in Japan. The severity of disease varies widely, from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma (HCC).[1,2] Eradication of HCV by antiviral treatment improves liver histology and patient survival.[3] A currently popular antiviral treatment regimen for the treatment of chronic HCV infection worldwide is pegylated interferon alpha (peg-IFN alpha) in combination with ribavirin (RBV). The combination treatment has resulted in a higher rate of sustained virological response (SVR), over 50% in Caucasian patients, than standard interferon (IFN) monotherapy.[4,5] However, there are no data concerning the response and safety of the combination treatment for a large number of Japanese patients with chronic HCV infection because this treatment was only approved by the Japanese Ministry of Health, Labor and Welfare in December 2004.

The HCV genotype has been reported to be the most important predictor of IFN treatment response.[4–14] Patients infected with genotypes 2 and 3 achieved approximately 65% SVR in a 24-week

Journal of Gastroenterology and Hepatology 23 (2008) 1094–1104

trial of non-peg-IFN alpha in combination with RBV, in contrast to patients with genotype 1 who had < 30% SVR.[13,14] SVR is also achieved consistently more often by patients with a low HCV-RNA level.[4–14] Moreover, host factors affect the chance of SVR, albeit less so than the genotype.[10] These factors include age, race, sex, obesity, and the degree of hepatic fibrosis and steatosis.[15] In a racial analysis, African Americans were shown to have response rates only one-half to one-third those of Caucasians.[15] In addition, Asian patients were more likely to achieve an SVR by treatment with peg-IFN alpha-2a and RBV than Caucasian patients.[16] The reasons for the racial differences in response rates to peg-IFN alpha plus RBV treatment are not well known.

Peg-IFN alpha was a substantive breakthrough in therapy because of the longer effect; the lasting, steady therapeutic blood level is a major pharmacokinetic advance.[4,5] The most frequent adverse effects during peg-IFN alpha plus RBV treatment are depression and hematological disorders such leukopenia, anemia, and thrombocytopenia. Therefore, the peg-IFN alpha plus RBV treatment often results in discontinuation or the need for a reduction of the dosage due to the adverse effects.

To investigate the efficacy and safety of antiviral treatments for Japanese chronic hepatitis B and C patients, a multicenter study, the Kyushu University Liver Disease Study (KULDS), was launched in 2003. Our group has previously reported several clinical studies.[17–21] The present report is a prospective, multicenter study carried out to analyze the association between the treatment length and the cumulative dose and effectiveness of peg-IFN alpha-2b plus RBV treatment for a large number of Japanese patients with chronic hepatitis C.

Methods

Patients

Treatment of chronic hepatitis C with a combination of peg-IFN alpha-2b and RBV was accepted by the Japanese Ministry of Health in October, 2004. A prospective study of 715 Japanese patients aged 18 years or older (586 and 129 patients with genotypes 1b and 2, respectively) treated with peg-IFN alpha-2b plus RBV between December 2004 and February 2007 who were all positive for antibody to HCV and HCV-RNA for over 6 months was carried out. The respective distribution rates were 82.0% and 18.0% for genotypes 1b and 2, similar to the reported epidemiological distribution.[22]

Criteria for exclusion were: (i) clinical or biochemical evidence of hepatic decompensation, advanced cirrhosis identified by large esophageal varices (F2 or F3), history of gastrointestinal bleeding, ascites, encephalopathy, or hepatocellular carcinoma; (ii) hemoglobin level < 115 g/L, white blood cell count $< 3 \times 10^9$/L, and platelet count $< 50 \times 10^9$/L; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen positive or HIV positive); (iv) excessive active alcohol consumption > 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy within 12 months prior to the enrollment. Patients who fulfilled the above criteria were recruited at Kyushu University Hospital and 32 affiliated hospitals in the northern Kyushu area of Japan.

Within the 3 months before the start of the treatment and every 3 months during the treatment period, each patient was tested for alpha-fetoprotein (AFP) and had abdominal ultrasonographic examination. If an abnormal AFP level of ≥ 40 ng/mL and/or an appearance of focal lesions at ultrasonographic examination was found at any testing, further testing for hepatocellular carcinoma (HCC) was done, which included dynamic computed tomography (CT), angiography, and/or tumor biopsy. Patients so confirmed to have HCC within 3 months after the start of the treatment were excluded from this study.

Table 1 shows the baseline characteristics of the enrolled patients. The median age was 58.0 years. Of the 715 patients, 198 (27.6%) were aged 65 years or over. In Japan, many older patients with chronic hepatitis C are candidates for antiviral treatment, different from many other countries. The rates of prior non-peg-IFN monotherapy significantly differed among the genotype-classified patients (genotype 1, 40.8% and genotype 2, 28.7%).

Table 1 Characteristics of 715 chronic hepatitis C patients treated with a combination of pegylated IFN alpha-2b and ribavirin, classified by HCV genotype

Characteristics	Total $n = 715$	Genotype 1 $n = 586$	Genotype 2 $n = 129$	*P*-value
Male *n* (%)	388 (54.3)	321 (54.8)	67 (51.9)	0.6250
Age (years)	56.8 ± 11.7	57.8 ± 10.3	52.6 ± 14.1	0.0004
Body mass index (kg/m^2)	23.4 ± 3.2	23.5 ± 3.1	23.5 ± 3.3	0.4999
Prior IFN monotherapy *n* (%)	276 (38.6)	239 (40.8)	37 (28.7)	0.0140
Prior combined IFN plus RBV treatment *n* (%)	69 (9.7)	60 (10.9)	5 (3.9)	0.0221
Alanine aminotransferase (IU/L)	77.1 ± 55.4	77.5 ± 52.8	70.9 ± 55.3	0.0594
γ-Glutamyltranspeptidase (IU/L)	60.6 ± 60.3	61.8 ± 58.6	50.8 ± 45.2	0.0241
Albumin (g/dL)	4.1 ± 0.4	4.1 ± 0.3	4.1 ± 0.3	0.1305
White blood cell (/mm^3)	5030.8 ± 1439.2	4993.0 ± 140.8	5260.6 ± 1658.2	0.3005
Hemoglobin (g/dL)	13.9 ± 1.4	13.9 ± 1.4	13.9 ± 1.5	0.7092
Platelet count (10^9/L)	165 ± 56	161 ± 52	185 ± 69	0.0013
Creatinine (mg/dL)	0.70 ± 0.16	0.70 ± 0.17	0.71 ± 0.16	0.1230
Creatinine clearance (mL/min)	97.9 ± 29.9	97.1 ± 29.8	101.3 ± 31.3	0.3621

Data are shown as the mean ± standard deviation.
HCV, hepatitis C virus; IFN, interferon; RBV, rivabirin.

Also, the rates of prior non-peg-IFN alpha plus RBV treatment significantly differed (genotype 1, 10.9% and genotype 2, 3.9%). These differences are explained by the necessity of re-treatment of patients with genotype 1 who had lower SVR by the standard IFN monotherapy than did non-genotype 1 patients, and because the RBV combination treatment with peg-IFN alpha-2b was approved in stages, first for patients with genotype 1 in October 2004, then for those with non-genotype 1 in January 2006. The means for age, platelet count, and γ-glutamyltranspeptidase (γ-GTP) in genotype 1 patients were significantly different than those of genotype 2 patients.

Informed consent was obtained from all patients before enrollment in this study. The study was approved by the institutional ethics committees of the hospitals involved and conducted in accordance with the ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization of guidelines for good clinical practice.

Treatment regimen

All patients were treated with a weight-based, 1.5 μg/kg weekly dose of subcutaneous peg-IFN alpha-2b (PegIntron A; Schering-Plough, Osaka, Japan). In combination with peg-IFN alpha-2b, RBV (Rebetol; Schering-Plough) was given orally at a daily dose of 600–1000 mg based on bodyweight (600 mg for patients weighing < 60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing ≥ 80 kg). The lengths of treatment were 48 weeks and 24 weeks for HCV genotypes 1 and 2 patients, respectively. The above durations and dosages are those approved by the Japanese Ministry of Health, Labor and Welfare. Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to < 100 g/L. In such cases, a reduction in the dose of RBV was required. Some patients also had peg-IFN alpha-2b-induced psychological adverse effects or a decrease of white blood cell and platelet count. In such cases, a reduction in the dosage of peg-IFN alpha-2b was required. Both peg-IFN alpha-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 85 g/L, 1×10^9/L, and 2.5×10^9/L, respectively. The treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic problems developed, continuation of treatment was judged not to be possible by the attending physician, or the patient desired discontinuation of treatment.

Clinical and laboratory assessment

Body mass index (BMI) was calculated as weight in kilograms/height in square meters. Blood samples were taken on enrollment, in the morning after 12 h overnight fasting. Serum levels of alanine aminotransferase (ALT), γ-GTP, white blood cell count, hemoglobin, and platelet count were measured by standard laboratory techniques at a commercial laboratory.

Determination of baseline HCV-RNA level and HCV genotype

The pretreatment, baseline, serum HCV-RNA level was measured by a quantitative HCV-RNA polymerase chain reaction (PCR) assay (COBAS Amplicor HCV Monitor Test v2.0 using the 10-fold dilution method; Roche Diagnostics, Tokyo, Japan), which has a lower limit of quantitation of 5000 IU (1350 copies)/mL (5 kIU/mL) and an outer limit of quantitation of 5 100 000 IU/mL (5100 kIU/mL). The HCV genotype was determined by a type-specific primer from the core region of the HCV genome. The protocol for genotyping was carried out as previously described.[23]

Efficacy of treatment

Sustained virological response was defined as serum HCV-RNA undetectable at 24 weeks after the end of treatment. Patients who had undetectable HCV-RNA within the initial 12 weeks of treatment were considered to have had an early virological response (EVR). These efficacy variables, SVR and EVR, were defined as non-detectable HCV-RNA as measured by the COBAS Amplicor HCV Monitor Test v2.0, and the results were labeled as positive or negative. The lower limit of detection was 50 IU/mL (0.5 kIU/mL). The analysis of SVR and EVR was done on an intention-to-treat basis.

Statistical analysis

Continuous data were expressed as mean values, the values ± standard deviation (SD), or the values ± standard error (SE) of the mean. The following statistics were done using a commercially available software package (BMDP Statistical Software Inc., Los Angeles, CA, USA) for the IBM 3090 system computer. The chi-squared or Fisher's exact test was used to examine the association between baseline characteristics and SVR. The Mann–Whitney *U*-test was also used to compare responders and non-responders with regard to various characteristics, when appropriate. The Cochran-Mantel-Haenszel test was used to test for statistical significance among the subgroups. A *P*-value of less than 0.05 was considered significant.

Results

Discontinuation of peg-IFN alpha-2b plus RBV treatment and adverse effects

Of the 715 patients, 152 (21.2%) did not complete peg-IFN alpha-2b plus RBV treatment due to adverse effects or for other reasons (Table 2). Although anemia, as a cause of discontinuation, was followed by general fatigue and depression, most patients discontinued the treatment because of general fatigue and depression together with anemia (hemoglobin 85–100 g/L).

The discontinuation rate was higher for patients with genotype 1 (138 of 586, 23.5%) than those with genotype 2 (14 of 129, 10.8%). The genotype 1 patients included 55 who stopped receiving treatment without virological effect (positive for serum HCV-RNA or no more than 2-$\log_{10}$ reduction from the pretreatment viral level) at 24 or more weeks after the start ($n = 22$), economic problems related to the high cost of treatment ($n = 6$), and other reasons (drop out, moving, nursing ill family members, and being arrested for a crime) ($n = 27$). Thus, the discontinuation rates for patients with adverse effects were only 14.1% (83 of 586) and 7.7% (10 of 129) for genotypes 1 and 2, respectively, with no significant difference. The majority were patients aged 65 years or

Table 2 Reasons for discontinuation of pegylated IFN plus ribavirin treatment, classified by HCV genotype

	Genotype 1	Genotype 2	Total
Adverse effects			
General fatigue	29	0	29
Depression	10	1	11
Encephalopathy	2	0	2
Anemia	11	0	11
Thrombocytopenia	1	1	2
Hyperthyroidism	5	1	6
Rash	6	3	9
Retinopathy	2	0	2
Interstitial pneumonia	1	1	2
Articular rheumatism	1	0	1
Brain infarction	0	1	1
Proteinuria	1	0	1
Hepatocellular carcinoma	11	2	13
Malignancy (extra-liver)†	2	0	2
Pulmonary tuberculosis	1	0	1
Other reasons			
No effect of treatment	22	2	24
Economic problems	6	0	6
Others‡	27	2	29
Total	138	14	152

†Includes one patient with gastric cancer and one patient with lung cancer.

‡Includes drop out ($n = 16$), patients who moved ($n = 6$), who nursed ill family members ($n = 3$), or who were arrested for criminal activity ($n = 2$).

over: 68 (73.1%) of the 93 discontinued due to adverse effects. The discontinuation rate due to adverse effects was significantly higher for patients aged 65 years or over (68 of 198, 34.3%) than for those aged under 65 years (25 of 517, 4.8%) ($P < 0.0001$). The mean times to discontinuation (± SD) were 23.0 ± 13.1 weeks and 20.2 ± 15.4 weeks for patients with genotypes 1 and 2, respectively.

SVR by intention-to-treat analysis

Of the 715 patients, 345 (48.2%) achieved SVR in the intention-to-treat analysis. SVR was significantly higher in genotype 2 (96 of 129, 74.4%) than in genotype 1 (249 of 586, 42.4%) ($P < 0.0001$). No significant differences in SVR were found between patients with and without prior non-peg-IFN monotherapy or non-peg-IFN plus RBV treatment between the genotype-classified patients.

An analysis of the association between SVR and the length of treatment showed that patients who completed the combination treatment had a significantly higher rate of SVR than did those with a shortened period of treatment (Fig. 1). Completing the 48-week combination treatment resulted in a significantly higher rate of SVR than either 1–11-week and 12–23-week treatments (both $P < 0.0001$), but there was no significant difference between 24 and 47 weeks and the complete 48 weeks of treatment ($P = 0.1260$). The SVR of patients with genotype 1 was significantly associated with a ≥ 24-week treatment period when compared with treatment < 24 weeks (244 of 481, 50.7% *vs* 5 of 105, 4.7%, $P < 0.0001$). In genotype 2 patients, SVR significantly increased with the treatment period: 28.6%, 57.1%, and 78.3% by 1–11-week, 12–23-week, and 24-week periods, respectively ($P = 0.0018$ by the Cochran-Mantel-Haenszel test).

The combination treatment was done for 443 (75.5%) and 110 (85.2%) of genotype 1 and 2 patients, respectively (Fig. 2). The rates of SVR for genotype 1 and 2 patients were significantly higher in those who continued the combination treatment than in those who discontinued RBV treatment: 230 of 443 (51.9%) versus 19 of 143 (13.2%) genotype 1 ($P < 0.0001$) and 89 of 110 (80.9%) versus seven of 19 (36.8%) genotype 2 ($P = 0.0002$). In genotype 1, 286 patients who required a reduced dosage during treatment (Groups B, C, and D) were able to complete the full 48 weeks of combination treatment. There were no significant differences in SVR among Groups A to D patients with genotypes 1 and 2. Of the patients who discontinued RBV treatment (143 with genotype 1 and 19 with genotype 2), most patients (138 (96.5%) with genotype 1 and 14 (73.7%) with genotype 2) did not complete combination treatment because there was no viral effect, because of adverse effects, or because they dropped out. The remaining patients discontinued the RBV treatment but completed the combination treatment without a reduction of the peg-IFN alpha-2b target dosage (three with genotype 1 and five with genotype 2), or discontinued the RBV treatment and completed their peg-IFN alpha-2b treatment with a reduction of the target dosage (two with genotype 1 and none with genotype 2).

An analysis of the association between SVR and the total dosage of peg-IFN alpha-2b and RBV during the treatment showed that patients with a higher total dosage of the combination treatment had a significantly higher rate of SVR than those with a lower dosage only for genotype 1 patients, although no significant difference was found in genotype 2 (Fig. 3). In genotype 1, reducing the total dosage of peg-IFN alpha-2b during the treatment significantly reduced the rate of SVR: 52.9% (187 of 353) for patients with ≥ 80% of the peg-IFN alpha-2b dosage, 36.5% (30 of 82) for those ≥ 60% but < 80% of the peg-IFN alpha dosage, and 21.1% (32 of 151) for those < 60% of the peg-IFN alpha dosage (both $P < 0.0001$). In genotype 1, the SVR rate of patients < 60% of the RBV dosage (91 of 307, 29.6%) was significantly lower than that of patients ≥ 80% of the RBV dosage (112 of 189, 59.2%) and those ≥ 60% but < 80% of the RBV dosage (46 of 90, 51.1%) (both $P < 0.0001$), although no significant difference was found between those ≥ 80% of the RBV dosage and those ≥ 60% but < 80% of the dosage. In genotype 2, neither a dosage reduction of peg-IFN nor RBV significantly influenced SVR.

An analysis of the association between SVR and the total combined dosage of peg-IFN alpha-2b plus RBV showed that patients with a higher total combined dosage of the combination treatment had a significantly higher rate of SVR than those with a lower dosage for genotype 1 patients only, although no significant difference was found in genotype 2 (Fig. 4). In genotype 1, the SVR rate of patients ≥ 80% of peg-IFN alpha-2b and ≥ 80% of RBV was significantly higher (78 of 122, 63.9%) than those without these combined dosages (171 of 464, 36.8%) ($P < 0.0001$). Moreover, the SVR rate of patients ≥ 80% of peg-IFN alpha-2b and ≥ 60% of RBV was significantly higher (116 of 187, 62.0%) than those without these dosages (133 of 399, 33.3%) ($P < 0.0001$). However, in genotype 2, neither a dosage reduction of peg-IFN nor RBV significantly influenced SVR.

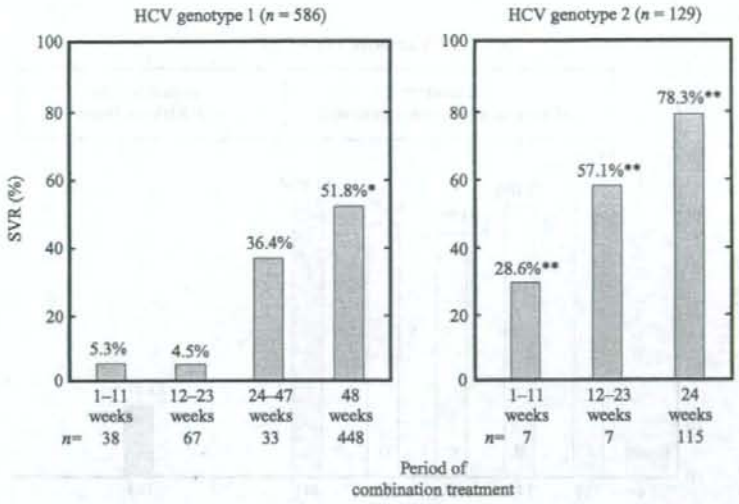

Figure 1 Sustained virological response (SVR) rates classified by length of pegylated interferon-alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) combination treatment and by hepatitis C virus (HCV) genotype: An intention-to-treat analysis.

Analysis of EVR and the first 12-week adherence

An EVR was significantly higher in patients with genotype 2 (119 of 129, 92.2%) than in those with genotype 1 (307 of 586, 52.3%) ($P < 0.0001$). An analysis of the association between SVR and EVR showed that patients with EVR had a significantly higher rate of SVR than did patients without EVR for both genotypes 1 and 2: 220 of 309 (71.1%) versus 29 of 277 (10.4%) in genotype 1, and 96 of 119 (80.6%) versus none of 10 (0%) in genotype 2 (all $P < 0.0001$).

An analysis of the association between EVR and the first 12-week combined dosage of peg-IFN alpha-2b plus RBV showed that patients with a higher total combined dosage of the combination treatment had a significantly higher rate of SVR than those with a lower dosage for genotype 1 patients only, although no significant difference was found in genotype 2 (Fig. 5). In genotype 1, the EVR rate of patients ≥ 80% of peg-IFN alpha-2b and ≥ 80% of RBV was significantly higher (217 of 357, 60.7%) than those without these dosages (92 of 229, 40.1%) ($P < 0.0001$). Moreover, the SVR rate of patients ≥ 80% of peg-IFN alpha-2b and ≥ 60% of RBV was significantly higher (262 of 445, 58.8%) than those without these dosages (47 of 141, 33.3%) ($P < 0.0001$). However, in genotype 2, neither a dosage reduction of peg-IFN nor RBV influenced EVR.

Discussion

To the best of our knowledge, no reports have been written on the efficacy and safety of peg-IFN alpha-2b plus RBV treatment for a large number of Japanese HCV patients. The present study by intention-to-treat analysis included over 700 Japanese patients with chronic hepatitis C, a sufficient number to provide a meaningful statistical analysis and to be of interest to clinical physicians. Our findings show that in peg-IFN alpha-2b plus RBV treatment for chronic hepatitis C it is important to complete the target treatment duration and to use the full dosage to achieve virological efficacy.

A recent study showed Asian patients with chronic hepatitis C were more likely to achieve an SVR by treatment with peg-IFN alpha-2a and RBV than were Caucasian patients, suggesting a genetic influence on the antiviral response.[16] A significant difference between Asian and Caucasian patients with genotype 1 infections (65% and 36%) was also reported. However, the study included only 52 Asian patients and had no analysis concerning dosage of peg-IFN and RBV. Because our study included a large number of Japanese patients and an analysis of the complete combination treatment and the dosage of peg-IFN alpha and RBV, the present study provides for meaningful statistical analysis.

Our analysis showed that the discontinuation of RBV was significantly associated with a marked decline in SVR. We also showed that a < 60% reduction of the total dosage was associated with a poor outcome. Several adverse reactions are strongly associated with RBV. One of the most significant problems is hemolytic, especially anemia.[14] Most patients with anemia have general fatigue. Careful administration is necessary for patients > 60 years old, female patients, and patients receiving an RBV dosage by bodyweight of ≥ 12 mg/kg.[24] In fact, most of our patients who required a reduction in the total dosage or who discontinued RBV had anemia or fatigue. Also, discontinuation in this study was frequently found in patients aged ≥ 65 years. In Japan, many older patients with chronic hepatitis C are candidates for antiviral treatment, different from other countries. It is important to reduce the dosage of RBV at an early a stage as possible to allow the safe continuation of the combination treatment, as shown by data that a reduction of up to 60% of the total dosage of RBV does not appear to adversely influence SVR in Japanese patients.

The duration and dose of antiviral treatment are the most important factors influencing treatment outcome, especially in

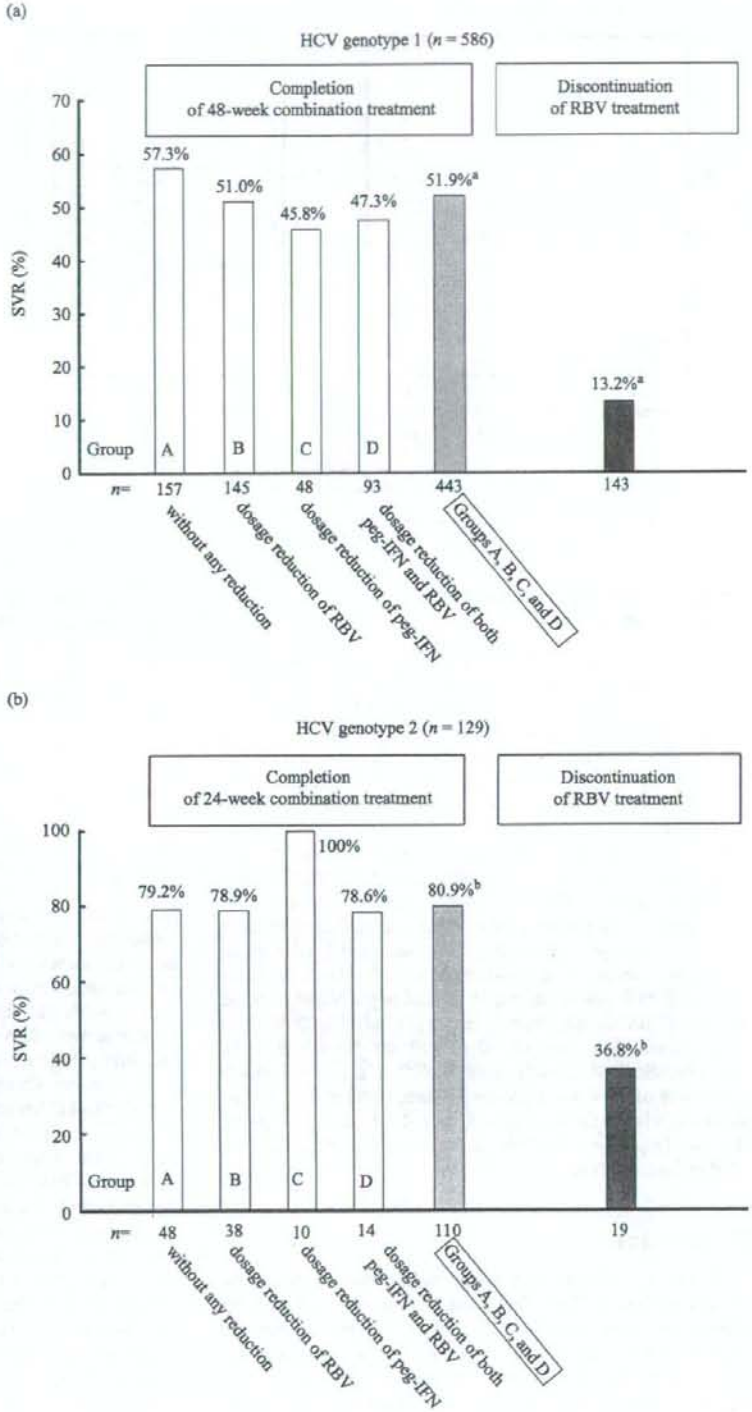

Figure 2 Sustained virological response (SVR) rates classified by continuation, reduction of the dosage, discontinuation of pegylated interferon-alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) treatment, and hepatitis C virus (HCV) genotype: An intention-to-treat analysis (Fig. 2a for genotype 1 and Fig. 2b for genotype 2). The following groups A, B, C, and D consisted of patients who completed their scheduled combination treatment (48 weeks for genotype 1 patients [$n = 443$] and 24 weeks for genotype 2 patients [$n = 110$]) and patients who discontinued RBV treatment (genotype 1 patients [$n = 143$] and genotype 2 patients [$n = 19$]). Group A patients well tolerated the combination treatment with peg-IFN alpha-2b and RBV without any reduction in the target dosage of either drug; Group B patients completed the combination treatment and had no reduction of peg-IFN alpha-2b dose, but needed a reduction of the RBV target dosage; Group C patients completed the combination treatment and had no reduction of RBV dosage, but needed a reduction of the target dosage of peg-IFN alpha-2b; Group D patients completed the combination treatment, but needed a reduction of the target dosage of both peg-IFN alpha-2b and RBV. 'a' and 'b' indicate significant differences between completion of the full combination treatment and discontinuation of RBV treatment ($P < 0.0001$ and $P = 0.0002$, respectively).

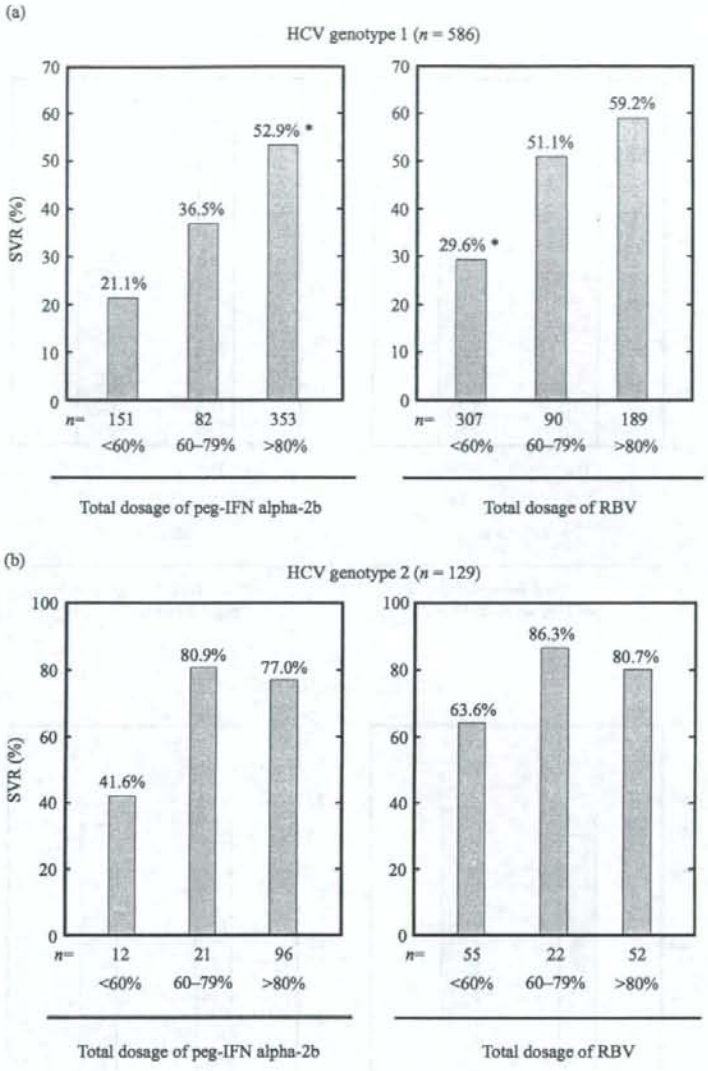

Figure 3 Sustained virological response (SVR) rates classified by percentage of total dosage of pegylated interferon-alpha2b (peg-IFN alpha-2b) plus ribavirin (RBV) treatment and by hepatitis C virus (HCV) genotype: An intention-to-treat analysis (Fig. 3a for genotype 1 and Fig. 3b for genotype 2). *indicates a significant difference between the groups.

HCV genotype 1-infected patients.[25,26] Shiffman and colleagues reported that reducing the total dose of peg-IFN alpha-2a to < 80% within the first 20 weeks of therapy significantly reduced SVR, but reducing the dose of RBV appeared to have little impact on SVR.[25] For our patients with genotype 1, the treatment period and total dosage were important to gaining SVR with peg-IFN alpha-2b plus RBV treatment. The 48-week combination treatment is the minimum requirement for SVR by these patients. Moreover, it is necessary to give ≥ 80% of the target dosage of peg-IFN alpha-2b (suitable for the weekly ≥ 0.9–1.2 μg/kg) and ≥ 60% of the target RBV (suitable for the daily 6–8 mg/kg) throughout the treatment.

Our previous report showed that a 24-week non-peg-IFN alpha plus RBV treatment regimen produced a high rate of SVR in Japanese genotype 2-infected patients.[19] The 24-week peg-IFN alpha-2b plus RBV treatment regimen used in the present study also demonstrated a remarkable rate of SVR (74.4%) for genotype 2 patients, as expected. This can be explained by the fact that genotype 2 patients have an extremely high rate of EVR, over 80%, with this combination treatment. Another important finding was that the total dosages of peg-IFN alpha-2b and RBV during the treatment for genotype 2 patients did not significantly influence SVR, although a dosage < 60% of the target resulted in a lower rate of SVR than a dosage ≥ 60%, without

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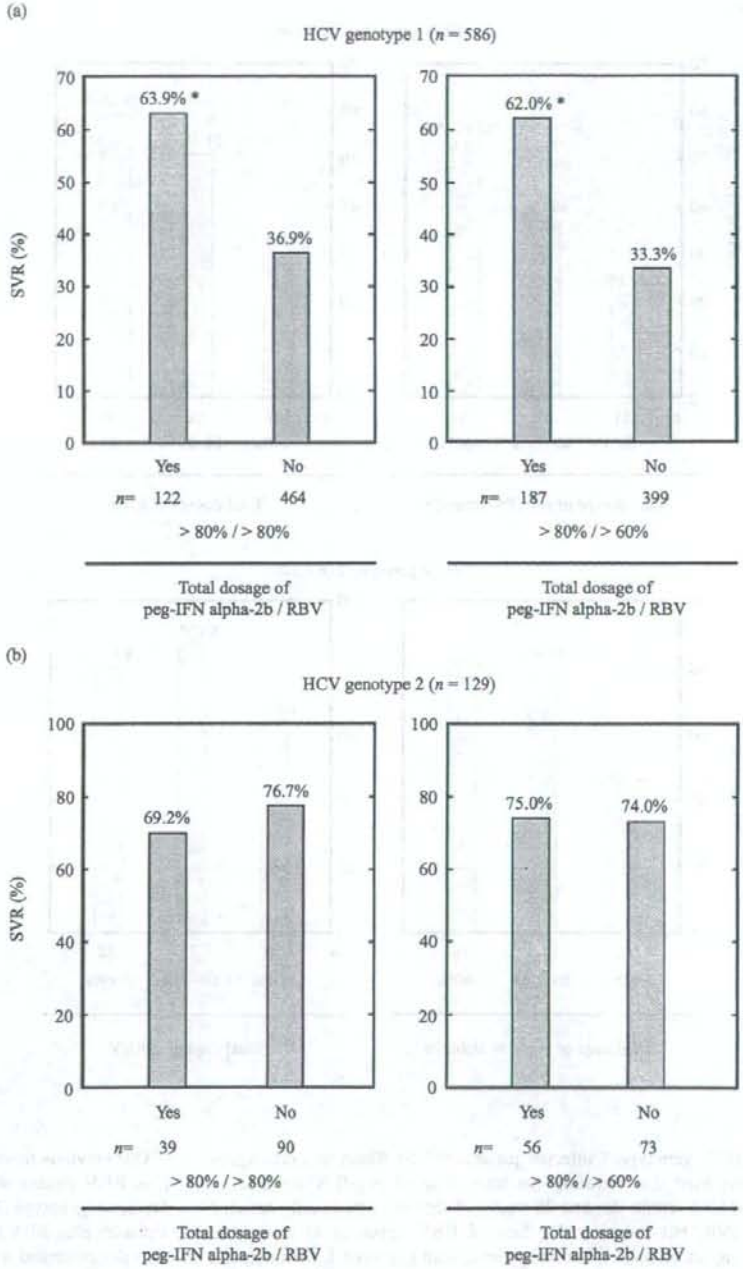

Figure 4 Sustained virological response (SVR) rates classified by percentage of total combined dosage of pegylated interferon alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) treatment and by hepatitis C virus (HCV) genotype: An intention-to-treat analysis (Fig. 4a for genotype 1 and Fig. 4b for genotype 2). *indicates a significant difference between the groups.

significant difference. These findings suggest that the target dosage can be reduced for genotype 2 patients to avoid the adverse effects such as general fatigue, depression, and anemia and that the 24-week combination treatment can still be successfully completed.

An EVR, a virological clearance by antiviral treatment in the initial 12 weeks, is significantly related with sustained response.[27] The present study also showed that the first 12-week combined dosage was significantly related with EVR in both genotype 1 and 2 patients, leading to the attainment of an SVR.

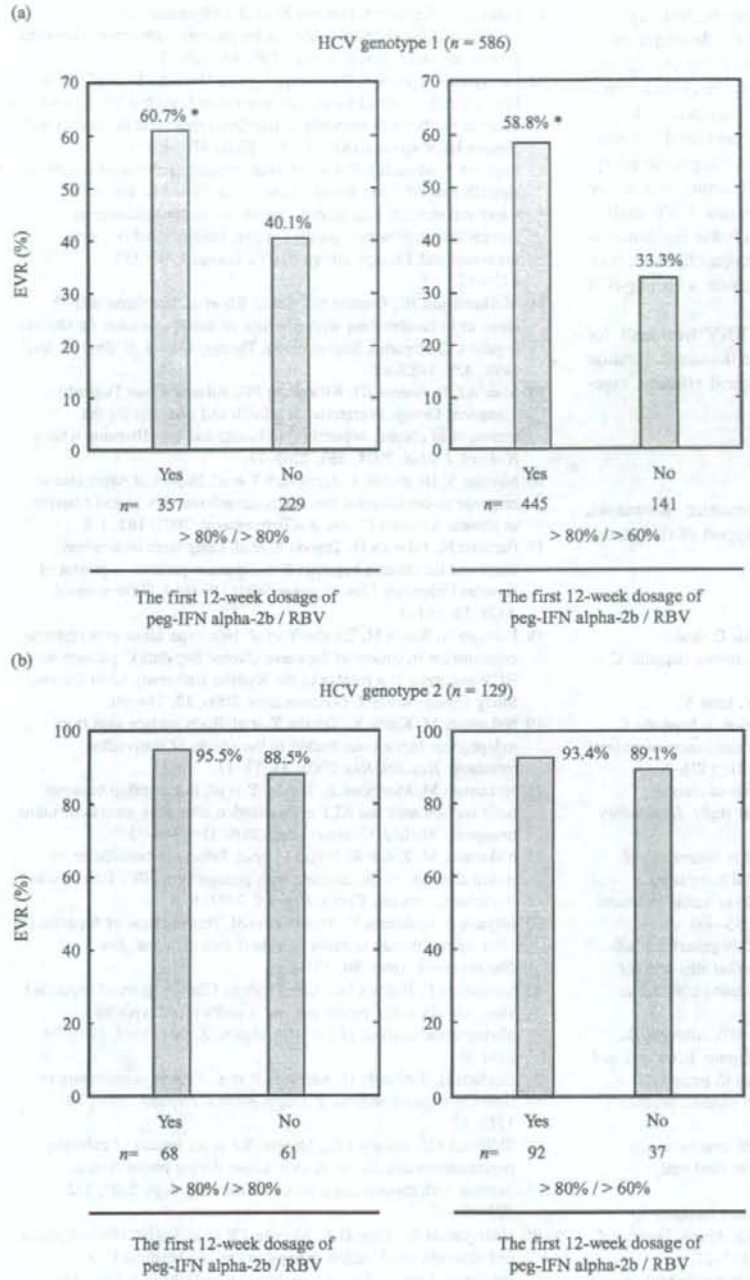

Figure 5 Early virological response (EVR) rates classified by percentage of the first 12-week combined dosage of pegylated interferon alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) treatment and by hepatitis C virus (HCV) genotype: An intention-to-treat analysis (Fig. 5a for genotype 1 and Fig. 5b for genotype 2). *indicates a significant difference between the groups.

Because of the impact of medical adherence during the first 12-week dosage on EVR, it is important to continue the dosage from the early stage to the target period in peg-IFN alpha-2b plus RBV treatment.

Since the introduction of peg-IFN alpha plus RBV combination regimen, the treatment of chronic hepatitis C has dramatically improved over the past decade and can cure a significant proportion of the patients.[5,6] However, the combination treatment has its

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limitations, especially for HCV genotype 1 patients. Although the limited efficacy and adverse effects necessitate the development of new therapeutics approaches, we must acknowledge the current situation in which many older Japanese patients with chronic hepatitis C are candidates for antiviral treatment. Therefore, a key to solving the problem is managing antiviral treatment for these older patients. Recent analysis suggests that using erythropoietic agents (epoetin and dartbepoetin) for the reduction of anemia may not be cost-effective for the majority of patients.[28] A new RBV analog, viramidine, is reported to be associated with a lower incidence of anemia than RBV (4% *vs* 27%),[29] and, if proven effective, may eventually be substituted for RBV in combination with peg-IFN alpha for patients with chronic hepatitis C.

In conclusion, in peg-IFN alpha-2b plus RBV treatment for chronic hepatitis C, it is important to complete the target duration and reach the target dosage to achieve virological efficacy, especially for genotype 1 patients.

Acknowledgments

We greatly thank Kazukuni Kawasaki, Tomohiro Kuwasaki, Naonori Ohe, and Toshihiro Ueda for their support of this study.

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Appendix I

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