peginterferon and ribavirin or combination therapy of peginterferon, ribavirin and protease inhibitor have a tendency to occur in elderly patients compared to young patients. Several authors have reported that interferon (IFN)-β plus ribavirin therapy might seem to have a strong effect and mild side-effects from reports of treatment to date.[12–14] This indicates the possibility that IFN-β plus ribavirin therapy could be given to elderly patients for eradication of HCV In particular, dose reduction might enhance the tolerability of IFN-β plus ribavirin therapy.

However, there is little information regarding efficacy of dose reduction in IFN-β plus ribavirin for elderly patients with chronic hepatitis C. Thus, in the present study, we performed a retrospective study to examine the efficacy of reduction therapy of IFN-β and ribavirin in elderly patients of 65 years or older with HCV genotype 1b and high viral load who had complications of anemia, low bodyweight (<50 kg), diabetes mellitus and/or hypertension.

METHODS

Patients

ELIGIBILITY CRITERIA FOR entry into the study included the following: (i) age of 65 years or older; (ii) HCV genotype 1b; (iii) serum level of HCV RNA of 5.0 logIU/mL or higher before treatment; (iv) no corticosteroid, immunosuppressive agents or antiviral agents used within 6 months; (v) no hepatitis B surface antigens, antinuclear antibodies or anti-mitochondrial antibodies detectable in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay or indirect immunofluorescence assay; (vi) leukocytes of more than 2000/mm^3, platelet count of more than 80 000/mm^3 and bilirubin of less than 2.0 mg/dL; (vii) follow up for more than 6 months before treatment; (viii) complication of anemia (hemoglobin <13 g/dL), low bodyweight (<50 kg), diabetes mellitus and/or hypertension. We excluded from the study all of the patients with the following: (i) a history of alcohol abuse; (ii) complication of malignancy; (iii) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices or ascites. From December 2007 to October 2010, a total of 23 HCV patients were enrolled in this retrospective cohort study at the study hospital. In these 23 patients, combination therapy was started with dose reduction of ribavirin. As control, another 22 patients without complications anemia, low bodyweight, and/or diabetes mellitus and/or hypertension treated with the standard dose of IFN-β and ribavirin were enrolled (standard-dose group). All collection and analysis of patient data for the dose-reduction group and standard-dose group was performed retrospectively from the patient records. This study had been approved by Institutional Review Board of our hospital.

Combination therapy of IFN-β and ribavirin

Treatment was provided for 48 weeks. IFN-β (Feron; Toray Industries, Tokyo, Japan) was administrated i.v. at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 44 weeks. Ribavirin (Rebetol; MSD, Whitehouse Station, NJ, USA) were given at the dose described based on bodyweight. In the standard-dose group, the ribavirin dose was adjusted according to bodyweight (600 mg for ≤60 kg, 800 mg for >60 kg and ≤80 kg, and 1000 mg for >80 kg). Twenty-two patients were given the standard dose of ribavirin as described above at the initiation of combination therapy (standard-dose group). On the other hand, 23 patients were given a reduced dose of ribavirin that decreased by one tablet per day compared to the standard group due to complications of having a hemoglobin level of less than 13 g/dL, bodyweight of less than 50 kg, diabetes and/or hypertension (reduction-dose group).

Aspartate aminotransferase to platelet ratio index (APRI) calculation method and prevalence of significant fibrosis

The hepatic fibrosis was evaluated by the APRI, which was calculated according to the following formula: APRI = (AST level / ULN) × 100 / platelet count (10^9/L), where ULN was the aspartate aminotransferase (AST) upper limit of normal (33 IU/L).

As previously reported, an APRI of more than 1.50 is predictive of significant fibrosis (positive predictive value, 88%; negative predictive value, 64%).[15]

Laboratory investigation

In this study, HCV RNA levels were evaluated at least once every month before, during and after therapy. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics, Basel, Switzerland). The linear dynamic range of the assay was 1.2–7.8 logIU/mL, and the undetectable samples were defined as negative. An SVR was defined as clearance of HCV RNA by COBAS TaqMan HCV test (Roche Diagnostics) at 6 months after the cessation of combination therapy.

Hepatitis C virus 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.[16] Inosine triphosphatase (*ITPA*) (rs1127354) and interleukin (*IL28B*) (rs8099917) were genotyped by the Invader assay (Third Wave Technologies, Madison, WI, USA), TaqMan assay or direct sequencing as described.[17–19] The core protein of HCV-1b was determined by the previous report.[20] Clinical evaluation and biochemical and hematological tests were performed at a minimum of 4-week intervals.

Statistical analysis

Non-parametric procedures were employed for the analysis of background features of the patients with and without SVR, including the Mann–Whitney *U*-test, Fisher's exact test and Kruskal–Wallis test. The following variables were evaluated as prognostic factors: sex, age, body mass index, a history of IFN therapy, a HCV RNA level, biochemical factors (AST, alanine aminotransferase, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol), platelet count, and HCV RNA 4, 8 and 12 weeks after the initiation of IFN therapy. Changes in hemoglobin, white blood cells and platelets between reduction-dose group and standard-dose group during follow up were analyzed by the Mann–Whitney *U*-test. Significance of trends in SVR based on adherence of IFN and ribavirin was determined with the Cochran–Armitage trend test. The SPSS software package (SPSS, Chicago, IL, USA) was used to perform statistical analysis. $P < 0.05$ was considered a statistically significant difference.

Table 1 Clinical backgrounds before combination therapy of IFN-β and ribavirin in chronic hepatitis c patients

Characteristic	Total	Reduction-dose group	Standard-dose group	*P*-value*
Patients, *n*	45	23	22	
Sex, male (%)	48.9%	30.4%	68.2%	0.017
Age (years)	67.5 ± 2.8	68.1 ± 2.6	66.9 ± 3.0	0.105
Height (cm)	159.4 ± 8.7	155.2 ± 6.6	163.6 ± 8.5	0.008
Weight (kg)	57.1 ± 8.7	54.1 ± 8.6	60.3 ± 7.7	0.017
BMI	22.6 ± 2.5	22.7 ± 2.9	22.5 ± 2.2	0.843
History of IFN (+)	60.0%	52.2%	68.2%	0.365
Diabetes (+/−)	2/43	2/21	0/22	0.489
Hypertension (+/−)	5/40	5/19	0/22	0.049
APRI	1.55 ± 1.22	1.39 ± 1.09	1.71 ± 1.34	0.619
APRI (≥1.5/<1.5)	22/23	10/13	12/10	0.556
HCV RNA (logIU/mL)	6.6 ± 0.6	6.6 ± 0.6	6.5 ± 0.5	0.712
IL28B (TT/TG)	34/11	19/4	15/7	0.314
HCV core 70 (wild/mutant)	31/14	17/6	14/8	0.530
ITPA (CC/CA)	31/14	14/9	17/5	0.337
AST (IU/L)	60 ± 36	58 ± 40	63 ± 33	0.555
ALT (IU/L)	89 ± 87	73 ± 79	109 ± 95	0.804
FPG (mg/dL)	107 ± 30	110 ± 37	105 ± 20	0.121
Triglyceride (mg/dL)	97 ± 41	87 ± 40	108 ± 41	0.073
Total cholesterol (mg/dL)	170 ± 28	164 ± 29	176 ± 27	0.193
HDL cholesterol (mg/dL)	46 ± 10	46 ± 11	46 ± 9	0.864
LDL cholesterol (mg/dL)	88 ± 33	84 ± 32	93 ± 35	0.479
Hemoglobin (g/dL)	13.7 ± 1.3	13.1 ± 1.1	14.4 ± 1.2	<0.001
WBC ($\times 10^3/mm^3$)	4.1 ± 1.1	4.3 ± 1.2	3.9 ± 0.9	0.354
Platelet ($\times 10^4/mm^3$)	15.2 ± 7 7	14.3 ± 5.4	16.2 ± 9.7	0.776

*Non-parametric procedures were employed for the analysis of background features of the patients in the reduction-dose group and the standard-dose group, including the Mann–Whitney *U*-test or Fisher's exact test.

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

ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon; IL, interleukin; ITPA, inosine triphosphatase; LDL, low density lipoprotein; WBC, white blood cell.

RESULT

Clinical characteristics of the patients

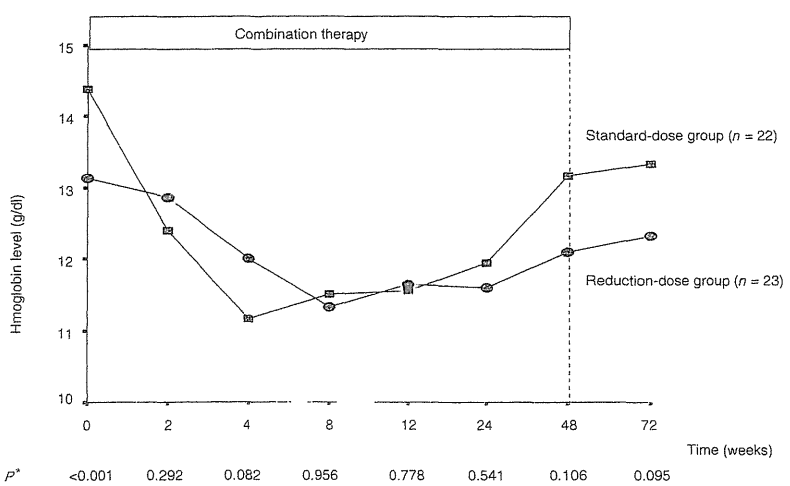
A TOTAL OF 45 patients were enrolled in the present study. Table 1 shows the characteristics before treatment of the elderly patients who received combination therapy. There were no significant differences in clinical backgrounds except for hemoglobin level, sex, height, bodyweight and hypertension between the reduction-dose group and standard-dose group.

Safety and tolerance of IFN

Of the 45 patients included in this study, nine of the patients discontinued combination therapy because of related adverse events (three patients) or poor response (six patients). In the reduction-dose group, one patient discontinued therapy at 8 weeks because of general fatigue and another two discontinued therapy because of poor response at 10 and 20 weeks. In the standard-dose group, two discontinued therapy at 3 and 12 weeks because of bronchitis and skin rash, respectively. Another four discontinued therapy because of poor response at 11, 13, 14 and 21 weeks.

Next, seven patients (four in the reduction-dose group and three in then standard-dose group) had dose reduction of IFN-β from 6 MU to 3 MU because of side-effects (five cases of thrombocytopenia and/or leukopenia, two cases of general fatigue). The onset of dose reduction based on IFN-related side-effects ranged 2–12 weeks after initiation of combination therapy. Moreover, 13 patients (three in the reduction-dose group and 10 in the standard-dose group) had further reduction of ribavirin due to anemia. Further reduction rate of ribavirin during treatment was 13% (3/23) in the reduction-dose group and 45% (10/22) in the standard-dose group. There was a statistically significant difference in further reduction rate of ribavirin between the reduction-dose group and the standard-dose group ($P = 0.008$). One patient of the reduction-dose group and two patients of the standard-dose group received both reduction of IFN-β and ribavirin during treatment.

Figure 1 shows the change of hemoglobin level after the initiation of combination therapy based on the difference between the reduction-dose group and standard-dose group. The hemoglobin level at the initiation of combination therapy in the reduction-dose group was statistically lower than that in the standard-dose group by the use of the Mann–Whitney *U*-test. However, there was no significant difference in the hemoglobin level between the reduction-dose group and the standard-dose group after the initiation of combination therapy. Figures 2 and 3 show the change of white blood cell and platelet levels after the initiation of combination therapy based on the difference between the reduction-dose group and the standard-dose group. There were no significant changes of average white blood cell and

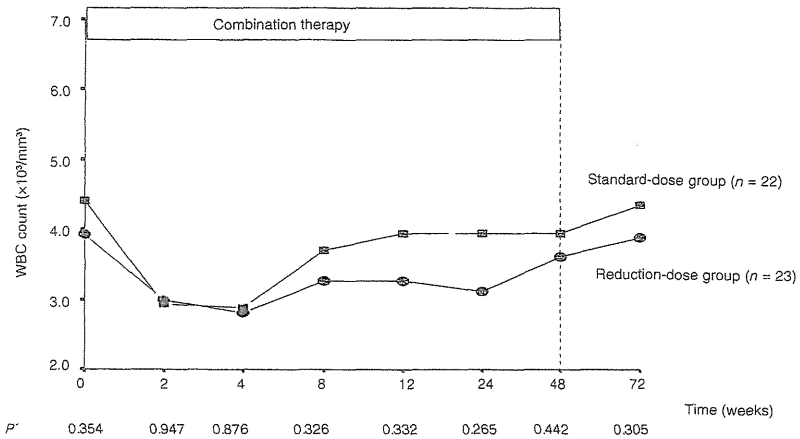


*P**	<0.001	0.292	0.082	0.956	0.778	0.541	0.106	0.095
Standard group†	0/22	2/22	7/21	8/20	10/18	10/16	10/16	
Reduction droup†	0/23	0/23	1/23	2/22	3/21	3/20	3/20	

*Statistical difference in hemoglobin level between reduction group and standard group

†No. of patients who were given new reduction of ribavirin dose during combination therapy/ total no. of patients who were given combination therapy

Figure 1 Change of hemoglobin level after the initiation of the combination therapy of interferon-β and ribavirin in the reduction-dose group and the standard-dose group.



*Statistical difference in white blood cell level between reduction-dose group and standard-dose group

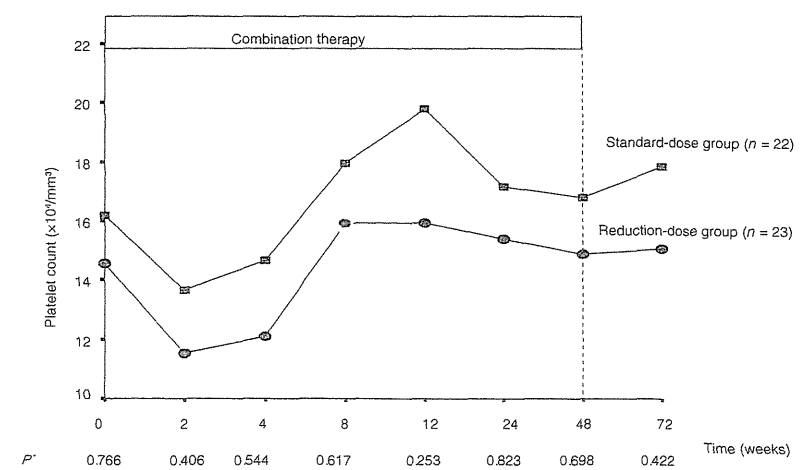
†No. of patients who were given new reduction of IFN-beta dose during combination therapy/ total no. of patients who were given combination therapy

Figure 2 Change of white blood cell count after the initiation of the combination therapy of interferon (IFN)-β and ribavirin in the reduction-dose group and the standard-dose group.

platelet levels during combination therapy between the reduction-dose group and the standard-dose group.

Efficacy of treatment

Out of the 45 patients enrolled in the present study, 15 patients (33.3%) achieved SVR by the intention-to-treat analysis. The SVR rate was 39.1% (9/23) in the reduction-dose group and 27.3% (6/22) in the standard-dose group. There was no significant difference in SVR rate between the reduction-dose group and the standard-dose group ($P = 0.404$). Table 2 shows the difference of clinical backgrounds between patients with and without SVR. On the predictive factor for SVR, the negativity of HCV RNA at 8–24 weeks after the initiation of treatment was an important factor. None of the patients with positive HCV RNA at 24 weeks after the



*Statistical difference in platelet level between reduction group and standard group

†No. of patients who were given new reduction of IFN-beta dose during combination therapy/ total no. of patients who were given combination therapy

Figure 3 Change of platelet count after the initiation of the combination therapy of interferon (IFN)-β and ribavirin in the reduction-dose group and the standard-dose group.

Table 2 Difference of clinical backgrounds between patients with SVR and those without SVR

	SVR ($n = 15$)	Non-SVR ($n = 30$)	*P*-value*
Age (years)	67.6 ± 2.4	67.5 ± 2.9	0.983
Sex (male/female)	5/10	16/14	0.340
Height (cm)	158.9 ± 10.1	159.6 ± 8.2	0.571
Weight (kg)	55.3 ± 5.8	57.8 ± 9.5	0.140
BMI	22.0 ± 2.3	22.9 ± 2.6	0.133
Diabetes (+/−)	0/15	2/28	0.545
Hypertension (+/−)	2/13	3/27	1.000
History of IFN (+/−)	6/9	21/9	0.105
HCV load (logU/mL)	6.5 ± 0.6	6.6 ± 0.5	0.572
APRI	1.15 ± 0.98	1.72 ± 1.29	0.140
IL28B (TT/TG)	15/0	19/11	0.008
HCV core 70 (wild/mutant)	11/4	20/10	0.743
ITPA (CC/CA)	9/6	22/8	0.497
AST (IU/L)	54 ± 28	63 ± 39	0.400
ALT (IU/L)	58 ± 27	73 ± 51	0.293
FPG (mg/dL)	106 ± 43	108 ± 23	0.197
Triglyceride (mg/dL)	99 ± 44	96 ± 41	0.255
Total cholesterol (mg/dL)	177 ± 24	167 ± 29	0.182
HDL cholesterol (mg/dL)	47 ± 9	45 ± 10	0.435
LDL cholesterol (mg/dL)	99 ± 31	84 ± 34	0.071
Hemoglobin (g/dL)	13.7 ± 1.3	13.5 ± 1.4	0.912
WBC ($\times 10^3/mm^3$)	3.9 ± 1.3	4.2 ± 0.9	0.525
Platelet ($\times 10^4/mm^3$)	19.4 ± 11.1	13.4 ± 5.1	0.012
HCV RNA (+/−) 4W	9/6	29/1	0.464
HCV RNA (+/−) 8W	6/9	28/2	0.021
HCV RNA (+/−) 12W	2/13	26/4	<0.001
HCV RNA (+/−) 24W	0/15	24/6	<0.001
Adherence of IFN (%)	89 ± 16	69 ± 31	0.009
Adherence of ribavirin (%)	77 ± 15	61 ± 27	0.064
Reduction group/standard group	9/6	14/16	0.404

*Non-parametric procedures were employed for the analysis of background features of the patients in the reduction-dose group and the standard-dose group, including the Mann–Whitney *U*-test or Fisher's exact test.
Data are number of patients (percentage) or mean ± standard deviation.
ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon; IL, interleukin; ITPA, inosine triphosphatase; LDL, low density lipoprotein; SVR, sustained virological response; W, weeks; WBC, white blood cell.

initiation of treatment achieved SVR. Based on genetic variations near the *IL28B* gene (rs8099917), SVR was 44.1% (15/34) in patients with TT and 0% (0/11) in patients with TG. SVR rate in patients with TT was significantly higher than that in patients with TG ($P = 0.008$). Regarding HCV core and *ITPA* gene, there was no significant difference between patients with SVR and patients without SVR.

Efficacy based on adherence

Tables 3–5 show the SVR rate based on adherence to combination therapy in the reduction-dose group, the standard-dose group and total patients. Patients with adherence of 2/3 or more for both IFN and ribavirin had an SVR of 40% or more in the reduction-dose group and the standard-dose group.

DISCUSSION

WE HAVE DESCRIBED the efficacy of reduction therapy of IFN-β and ribavirin in elderly patients infected with HCV genotype 1b and high viral load. Several findings from the present study have direct implications for combination therapy for elderly patients with HCV genotype 1b and high viral load in the future.

Table 3 Sustained virological response rate based on adherence of combination therapy in the reduction-dose group

Ribavirin dose	β-Interferon			Total†
	<1/3	≥1/3–<2/3	≥2/3	
<1/3	0% (0/2)	None	None	0% (0/2)
≥1/3–<2/3	None	0% (0/2)	50% (1/2)	25% (1/4)
≥2/3	None	33% (1/3)	50% (7/14)	47% (8/17)
Total*	0% (0/2)	20% (1/5)	50% (8/16)	39% (9/23)

*$P = 0.046$ for comparison of the three interferon groups (Cochran–Armitage trend test).
†$P = 0.075$ for comparison of the three ribavirin groups (Cochran–Armitage trend test).

Table 4 Sustained virological response rate based on adherence of combination therapy in the standard-dose group

Ribavirin dose	β-Interferon			Total†
	<1/3	≥1/3–<2/3	≥2/3	
<1/3	0% (0/3)	None	None	0% (0/3)
≥1/3–<2/3	None	0% (0/2)	0% (0/3)	0% (0/5)
≥2/3	None	50% (1/2)	42% (5/12)	43% (6/14)
Total*	0% (0/3)	25% (1/4)	33% (5/15)	27% (6/22)

*$P = 0.130$ for comparison of the three interferon groups (Cochran–Armitage trend test).
†$P = 0.024$ for comparison of the 3 ribavirin groups (Cochran–Armitage trend test).

First, the dropout rate due to side-effects in combination therapy of IFN-β and ribavirin in elderly patients with aged 65 years or older was 4.3% (1/23) in the reduction-dose group and 9.1% (2/22) in the standard-dose group. In the previous study, we reported that 68 of 612 patients treated with peginterferon and ribavirin stopped the treatment due to side-effects and the dropout rate was 14.9% in 1 year.[9] Although the 612 patients treated with peginterferon and ribavirin had a mean age of 53 years, the dropout rate tended to be high compared to combination therapy of IFN-β and ribavirin for elderly patients. This means that combination therapy of IFN-β and ribavirin might be safe compared with combination therapy of peginterferon and ribavirin. However, in the present study, the ratio of patients treated with the scheduled dose was approximately 23% in the standard-dose group. Most patients received reduction of drugs at the initiation of combination therapy or during combination therapy. Thus, physicians in charge should particularly pay attention to onset of treatment-induced side-effects in combination therapy for elderly patients.

Second, 15 out of 45 patients achieved SVR. When patients with genotype 1b and high viral load have been treated with IFN-β monotherapy, it has been reported that the SVR rate ranges 0–11%.[12,21] Thus, the present study indicates that the combination therapy of IFN-β and ribavirin is more effective for elderly patients with HCV genotype 1b and high viral load compared with IFN-β monotherapy.

Table 5 Sustained virological response rate based on adherence of combination therapy in the total patients

Ribavirin dose	β-Interferon			Total†
	<1/3	≥1/3–<2/3	≥2/3	
<1/3	0% (0/5)	None	None	0% (0/5)
≥1/3–<2/3	None	0% (0/4)	20% (1/5)	11% (1/9)
≥2/3	None	40% (2/5)	46% (12/26)	45% (14/31)
Total*	0% (0/5)	22% (2/9)	42% (13/31)	33% (15/45)

*$P = 0.022$ for comparison of the three interferon groups (Cochran–Armitage trend test).
†$P = 0.007$ for comparison of the 3 ribavirin groups (Cochran–Armitage trend test).

Third, the negativity of HCV RNA at 8–24 weeks after the initiation of treatment was an important factor for predicting SVR. None of the patients with positive HCV RNA at 24 weeks after the initiation of treatment achieved SVR. This result shows that negative HCV RNA at 24 weeks after the initiation of treatment could be a predictive marker for eliminating the HCV by combination therapy of IFN-β and ribavirin for 48 weeks.

Fourth, patients with adherence of 2/3 or more for both IFN and ribavirin had SVR of 40% or more in both the reduction-dose group and the standard-dose group. Seventeen of 22 patients in the standard-dose group had dose reduction or discontinuation of treatment. On the other hand, six of 23 patients in the reduction-dose group had dose reduction or discontinuation of treatment. Thus, many patients in the standard-dose group did not receive the dose of IFN and/or ribavirin as scheduled. Our results suggests that adherence of 2/3 or more for both IFN and ribavirin might enhance the elimination of HCV.

Fifth, based on genetic variations near the *IL28B* gene (rs8099917), SVR was approximately 45% in patients with TT. On the other hand, our result shows that SVR was rare in patients with TG. This result suggests that elderly patients with HCV genotype 1b, high viral load and IL28B gene (rs8099917) of TG should avoid combination therapy of IFN-β and ribavirin because of poor clearance of HCV.

Finally, there was no significant difference in the complete blood cell count between the reduction-dose group and the standard-dose group during combination therapy. In the standard-dose group, many patients discontinued the combination therapy or received dose reduction as described above. The further reduction of ribavirin or discontinuation of treatment might produce elevation of the hemoglobin level at 48 weeks after the initiation of combination therapy in the standard-dose group.

The present study was limited to patients with genotype 1b and HCV load of 5.0 logIU/mL or more. Moreover, in 40 of 45 patients histological examination of the liver was not undertaken within 1 year before combination therapy. In the present study, we tried to evaluate liver fibrosis by the APRI.[15] Our results show that SVR was not statistically associated with the APRI. In the present study, unfortunately, we checked HCV mutations in the core region and IFN sensitivity-determining region in only a few patients. Thus, we could not discuss the relationship between HCV mutation and SVR in the present study. Another limitation is that the present study was not a randomized controlled study.

β-Interferon is inconvenient for treatment compared to i.m. or s.c. injection. However, IFN-β-related side-effects are mild and few compared to combination therapy of IFN-α.[8,9] In fact, IFN-β-induced mental disorders are mild compared to those induced by IFN-α.[22] Moreover, IFN-β could be given in elderly patients of 70 years or older because of mild side-effects.[23] Additionally, platelet count recovered to the baseline at 12–48 weeks after the initiation of combination therapy.[24] Thus, combination therapy of IFN-β and ribavirin might be given to patients such as the elderly and/or slightly depressive.

In conclusion, the reduction therapy of IFN-β and ribavirin in elderly HCV patients with genotype 1b, high viral load and IL28B gene (rs8099917) of TT who had complications of anemia, low bodyweight, diabetes mellitus and/or hypertension is one possible selection of treatment.

ACKNOWLEDGMENTS

THE PRESENT WORK was supported in part by Grants-in-Aid from the Japanese Ministry of Health, Labor and Welfare. The authors acknowledge the editorial assistance of Thomas Hughes.

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Journal of Medical Virology 84:1593–1599 (2012)

Association of *IL28B* Genotype and Viral Response of Hepatitis C Virus Genotype 2 to Interferon Plus Ribavirin Combination Therapy

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The impacts of IL28B genotype to treatment response of hepatitis C virus (HCV) genotype 2 are still not clear. A total of 381 consecutive Japanese patients infected with HCV genotype 2, who could complete combination therapy with interferon (IFN) plus ribavirin for 24 weeks, were evaluated to investigate pretreatment predictors. Patients, who could not achieve sustained virological response at the first course of 24-week IFN plus ribavirin, were recruited into the study protocol of total 48-week IFN plus ribavirin. In 24-week regimen, rates of sustained virological response and rapid virological response were 82% and 50%, respectively. There were no significant differences in rates of sustained virological response and rapid virological response, according to IL28B genotype. Multivariate analysis identified younger age, higher level of albumin, absence of past history of IFN, and lower level of viremia as significant determinants of sustained virological response. As significant or marginal significant determinants of non-sustained virological response regardless of rapid virological response, multivariate analysis identified IL28B rs8099917 genotype TG + GG and lower level of albumin. In 48-week regimen to 10 patients of non-sustained virological response at the first course of 24-week regimen, sustained virological response rates were 70%. All of six patients, with IL28B TT and relapse at the first course of 24-week regimen, could achieve sustained virological response, but two patients with IL28B TG could not achieve sustained virological response. In conclusion, the present results suggest that IL28B genotype might partly affect viral response of HCV genotype 2 to combination therapy. ***J. Med. Virol. 84:1593–1599, 2012.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: HCV; IL28B; genotype 2; interferon; ribavirin; sustained virological response

INTRODUCTION

The response to interferon (IFN)-based therapy varies according to hepatitis C virus (HCV) genotype [Simmonds, 1997; Haydon et al., 1998]. In Japan, about 70% of patients with chronic hepatitis C are infected with HCV genotype 1b (HCV-1b), and about 30% are HCV genotype 2a or 2b (HCV-2a/2b) [Akuta et al., 2002]. Sustained virological response to 48-week IFN plus ribavirin combination therapy is about 50% in HCV-1b infection, and sustained virological response to 24-week combination therapy is more than 80% in HCV-2 infection [Manns et al., 2001; Fried et al., 2002; Mangia et al., 2005, 2009; von Wagner et al., 2005; Fujiwara et al., 2006].

IFN plus ribavirin combination therapy carries potential serious side effects and is costly especially when used long enough to achieve a high sustained virological response. For these reasons, especially in HCV-2 infection, it is needed to identify those patients who could achieve sustained virological response with shorter treatment course (16 weeks or less) to free them of unnecessary side effects and reduce costs,

Grant sponsor: Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan.

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Accepted 14 June 2012
DOI 10.1002/jmv.23368
Published online in Wiley Online Library (wileyonlinelibrary.com).

preferably as early as possible [Mangia et al., 2005, 2009; von Wagner et al., 2005]. However, the suitable treatment duration, based on the consideration of risk/benefit and cost/benefit, is still unclear in patients infected with HCV-2.

Furthermore, IL28B genotype is a pretreatment predictor of virological response to PEG-IFN/ribavirin dual therapy or telaprevir/PEG-IFN/ribavirin triple therapy in patients infected with HCV-1 [Ge et al., 2009; Tanaka et al., 2009; Suppiah et al., 2009; Akuta et al., 2010a]. Recent studies have investigated the effect of IL28B genotype on treatment efficacy to PEG-IFN/ribavirin combination therapy in cohort including HCV-2 patients [Rauch et al., 2010; Mangia et al., 2010; Kawaoka et al., 2011; Sakamoto et al., 2011], but it is not clear at this stage whether IL28B genotype can be used to predict the virological response to HCV-2.

The present study included 381 Japanese patients with infected HCV-2, who could complete a total of 24 weeks of IFN plus ribavirin combination therapy. The aims of the study were to investigate pretreatment predictive factors including IL28B genotype and the extending combination therapy with IFN plus ribavirin for HCV-2.

PATIENTS AND METHODS

Patients and Study Design

A total of 517 HCV genotype 2 (HCV-2)-infected Japanese patients were consecutively recruited into the study protocol of the combination therapy with IFN (PEG-IFNα-2b, IFNα-2b, or IFNβ) plus ribavirin for 24 weeks between March 2002 and February 2011 at Toranomon Hospital, Tokyo, Japan. Among these, 381 patients, who could complete a total of 24 weeks of combination therapy, were enrolled in this retrospective study and fulfilled the following criteria: (1) They were negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan). (2) They were naive to ribavirin therapy. (3) They were infected with HCV-2a or HCV-2b alone, confirmed by sequence analysis. (4) Absence of decompensated liver cirrhosis and hepatocellular carcinoma. (5) All were free of coinfection with human immunodeficiency virus. (6) None had been treated with antiviral or immunosuppressive agents within the preceding 3 months of enrolment. (7) None was an alcoholic; lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (8) None had other forms of hepatitis, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (9) They consented to the study, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected by approval by the human ethics review committee. They were evaluated the rates of sustained virological response (HCV-RNA undetectable at 24 weeks after the completion of therapy), rapid virological response (HCV-RNA undetectable at 4 weeks after the commencement of therapy), and non-response (HCV-RNA detectable during or at the end of therapy), based on the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). Furthermore, pretreatment predictors of treatment efficacy were investigated in 24-week regimen with IFN plus ribavirin combination therapy. Furthermore, patients, who could not achieve sustained virological response at the first course of 24-week regimen, were recruited into the study protocol of total 48-week combination therapy with IFN plus ribavirin. The decision to receive 48-week regimen was made by the patient, and they were evaluated treatment efficacy of extending combination therapy with IFN plus ribavirin.

Table I summarizes the profiles and data of the 381 patients at the commencement of 24-week combination therapy with IFN plus ribavirin. They included 188 men and 193 women, aged 15–76 years (median, 55 years). In all patients, the total duration of treatment was 24 weeks. In 107 of the 381 (28.1%) patients, the dose of ribavirin was reduced during treatment due to a fall in Hb concentration. With regard to the treatment protocol, 266 (69.8%) patients received PEG-IFNα-2b plus ribavirin for 24 weeks, and the remaining 115 (30.2%) patients received IFNα-2b or IFNβ plus ribavirin for 24 weeks. They received PEG-IFNα-2b at a median dose of 1.5 μg/kg (range, 0.6–1.9 μg/kg) subcutaneously each week, or IFNα-2b or IFNβ at a median dose of 6 million units (range, 3–6 million units) intramuscularly each day (seven times per week for initial 2 or 4 weeks, followed by three times per week for 24 weeks). They also received oral ribavirin at a median dose of 11.3 mg/kg (range, 3.1–15.3 mg/kg) daily.

Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment and were analyzed for levels of alanine aminotransferase and HCV-RNA. The serum samples were frozen at –80°C within 4 hr of collection and thawed at the time of measurement. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of NS5 region [Chayama et al., 1993]. HCV-RNA levels were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml.

Determination of IL28B and ITPA Genotype

IL28B (rs8099917) and ITPA (rs1127354) were genotyped by the Invader assay, TaqMan assay, or direct sequencing, as described previously [Ohnishi et al., 2001; Suzuki et al., 2003, 2011].

Statistical Analysis

Non-parametric tests (Chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate

TABLE I. Patient Profile and Laboratory Data at Commencement of 24-Week Combination Therapy of Interferon Plus Ribavirin in 381 Patients Infected With HCV Genotype 2

Demographic data	
Number of patients	381
Sex (male/female)	188/193
Age (years)*	55 (15–76)
History of blood transfusion	134 (35%)
Family history of liver disease	79 (21%)
Body mass index (kg/m^2)*	22.5 (14.6–37.8)
Laboratory data*	
HCV genotype (2a/2b)	238/143
Level of viremia (log IU/ml)	6.2 (1.5–7.5)
Serum aspartate aminotransferase (IU/L)	39 (7–404)
Serum alanine aminotransferase (IU/L)	48 (8–825)
Serum albumin (g/dl)	3.8 (2.9–4.7)
Gamma-glutamyl transpeptidase (IU/L)	32 (6–476)
Leukocytes (mm^3)	4,800 (2,100–10,400)
Hemoglobin (g/dl)	14.0 (9.9–19.1)
Platelet count ($\times 10^4/mm^3$)	18.1 (6.1–35.7)
Alpha-fetoprotein (μg/L)	4 (2–214)
Uric acid (mg/dl)	5.3 (2.2–9.4)
Serum ferritin (μg/L)	118 (10–1,305)
Total cholesterol (mg/dl)	178 (107–341)
Triglycerides (mg/dl)	93 (34–1,062)
High-density lipoprotein cholesterol (mg/dl)	50 (15–109)
Low-density lipoprotein cholesterol (mg/dl)	105 (18–245)
Fasting plasma glucose (mg/dl)	92 (69–187)
Indocyanine green retention rate at 15 min (%)	13 (3–39)
IL28B genotype	
rs8099917 genotype (TT/TG/GG)	147/46/1
ITPA genotype	
rs 1127354 genotype (CC/CA/AA)	121/37/6
Treatment	
PEG-IFNα-2b/IFNα-2b/IFNβ	266/70/45
Ribavirin dose (mg/kg)*	11.3 (3.1–15.3)
Past history of IFN monotherapy	114 (30%)

Data are number and percentages of patients, except those denoted by *, which represent the median (range) values.

logistic regression analyses were used to determine the factors that significantly contributed to treatment efficacy. The odds ratios and 95% confidence intervals (95% CI) were also calculated. All *P*-values less than 0.05, and 0.1 by the two-tailed test were considered significance ($P < 0.05$) and marginal significance ($P < 0.1$), respectively. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. Potential predictive factors associated with treatment efficacy included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, HCV genotype, level of viremia, serum aspartate aminotransferase, alanine aminotransferase, serum albumin, gamma-glutamyl transpeptidase, leukocytes, hemoglobin, platelet counts, alpha-fetoprotein, uric acid, serum ferritin, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting plasma glucose, indocyanine green retention rate at 15 min, IL28B and ITPA genotype, type of IFN (PEG-IFNα-2b, IFNα-2b, or IFNβ), ribavirin dose/body weight, and past history of IFN monotherapy. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, IL).

RESULTS

Virological Response Rates by 24-Week Combination Therapy

Sustained virological response was achieved by 311 of 381 (81.6%) patients, and rapid virological response by 188 of 378 (49.7%). 14 of 188 (7.4%) patients could not achieve sustained virological response regardless of rapid virological response. Only 14 of 381 (3.7%) patients were considered non-response. According to type of IFN, the sustained virological response rate was not significantly different among PEG-IFNα-2b (219 of 266 [82.3%] patients), IFNα-2b (56 of 70 [80.0%]), and IFNβ (36 of 45 [80.0%]).

Table II indicates treatment efficacy, according to IL28B rs8099917 genotype. Association of IL28B genotype and viral response could be evaluated in 193 patients. There were no significant differences in rates of sustained virological response, rapid virological response, and non-response, according to IL28B genotype (TT vs. TG + GG). In patients of HCV-2a or HCV-2b, there were also no significant differences in rates of treatment response, according to IL28B genotype.

TABLE II. Treatment Efficacy to Combination Therapy With Interferon Plus Ribavirin for 24 Weeks in Patients Infected With HCV Genotype 2, According to *IL28B* rs8099917 Genotype

	All cases	Genotype 2a	Genotype 2b
Sustained virological response (%)	n = 193	n = 117	n = 76
TT	71% (104/146)	74% (64/87)	68% (40/59)
TG + GG	72% (34/47)	73% (22/30)	71% (12/17)
P^*(TT vs. TG + GG)	$P = 1.000$	$P = 1.000$	$P = 1.000$
Rapid virological response (%)	n = 192	n = 117	n = 75
TT	48% (70/145)	51% (44/87)	45% (26/58)
TG + GG	36% (17/47)	43% (13/30)	24% (4/17)
P^*(TT vs. TG + GG)	$P = 0.178$	$P = 0.531$	$P = 0.161$
Non-response (%)	n = 193	n = 117	n = 76
TT	6% (9/146)	8% (7/87)	3% (2/59)
TG + GG	4% (2/47)	3% (1/30)	6% (1/17)
P^*(TT vs. TG + GG)	$P = 1.000$	$P = 0.678$	$P = 0.538$

Sustained virological response: HCV-RNA undetectable at 24 weeks after the completion of therapy. Rapid virological response: HCV-RNA undetectable at 4 weeks after the commencement of therapy. Non-response: HCV-RNA detectable during or at the end of therapy. Of 55 patients, 11, who could not achieve sustained virological response, were considered non-response.
*Evaluated by Chi-squared test or Fisher's exact probability test.

Predictive Factors Associated With Sustained Virological Response by 24-Week Combination Therapy in Multivariate Analysis

Univariate analysis identified six parameters associated with sustained virological response that achieved statistical significance. These included age (<50 years; $P < 0.001$), serum albumin (≥3.9 g/dl; $P < 0.001$), indocyanine green retention rate at 15 min (<15%; $P = 0.002$), past history of IFN monotherpy (absent; $P = 0.002$), level of viremia (<6.0 log IU/ml; $P = 0.010$), and history of blood transfusion (absent; $P = 0.036$).

Multivariate analysis identified four parameters that independently influenced sustained virological response, including age (<50 years; $P = 0.001$), serum albumin (≥3.9 g/dl; $P = 0.002$), past history of IFN monotherpy (absent; $P = 0.020$), and level of viremia (<6.0 log IU/ml; $P = 0.035$) (Table III).

Predictive Factors Associated With Non-Sustained Virological Response, Regardless of Rapid Virological Response, by 24-Week Combination Therapy in Multivariate Analysis

Univariate analysis identified four parameters associated with non-sustained virological response regardless of rapid virological response that achieved statistical significance. These included age (≥55 years; $P = 0.001$), serum albumin (<3.9 g/dl; $P = 0.002$), indocyanine green retention rate at 15 min (≥15%; $P = 0.009$), and IL28B genotype (TG + GG; $P = 0.036$).

Multivariate analysis identified two parameters that independently influenced non-sustained virological response regardless of rapid virological response, including IL28B genotype (TG + GG; $P = 0.017$), and serum albumin (<3.9 g/dl; $P = 0.084$) (Table IV).

Virological Response Rates by 48-Week Combination Therapy

Of 70 patients, 10 who could not achieve sustained virological response at the first course of 24-week regimen, were recruited into the study protocol of total 48-week combination therapy with IFN plus ribavirin. Table V summarizes the characteristics of the 10 patients at the commencement of the second course combination therapy with IFN plus ribavirin. They included six men and four women, aged 40–67 years (median, 57 years). Four cases were HCV-2a and the other six cases were HCV-2b. They received PEG-IFNα-2b at a median dose of 1.4 μg/kg (range, 1.1–1.7 μg/kg) subcutaneously each week. They also received oral ribavirin at a median dose of 10.6 mg/kg (range, 7.0–12.6 mg/kg) daily.

TABLE III. Factors Associated With Sustained Virological Response to Combination Therapy With Interferon Plus Ribavirin for 24 Weeks in Patients Infected With HCV Genotype 2, Identified by Multivariate Analysis

Factors	Category	Odds ratio (95% CI)	*P*
Age (years)	1: ≥50	1	
	2:<50	3.95 (1.76–8.85)	0.001
Serum albumin (g/dl)	1: <3.9	1	
	2: ≥3.9	2.80 (1.48–5.30)	0.002
Past history of interferon monotherapy	1: Present	1	
	2: Absent	2.08 (1.12–3.85)	0.020
Level of viremia (log IU/ml)	1: ≥6.0	1	
	2: <6.0	2.05 (1.05–4.00)	0.035

Only variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.010$) on multivariate logistic regression are shown.

TABLE IV. Factors Associated With Non-Sustained Virological Response in Patients, Who Achieved Rapid Virological Response to Combination Therapy With Interferon Plus Ribavirin for 24 Weeks in Patients Infected With HCV Genotype 2, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	*P*
IL28B rs8099917 genotype	1: TT	1	0.001
	2: TG + GG	3.95 (1.76–8.85)	
Serum albumin (g/dl)	1: ≥3.9	1	0.084
	2: <3.9	5.26 (0.80–34.5)	

Only variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on multivariate logistic regression are shown. Of 188 patients, 14, who could achieve rapid virological response, were considered non-sustained virological response.

Sustained virological response was achieved by 7 of 10 patients (70%). One patient was relapse (HCV-RNA undetectable at the end of therapy, and detectable at 24 weeks after the completion of therapy), and two patients were considered non-response. All of six patients, with IL28B TT and relapse at the first course of 24-week regimen, could achieve sustained virological response. Furthermore, two patients with IL28B TG could not achieve sustained virological response. Interestingly, one patient (Case 7), with IL28B TG regardless of relapse at the first course, could not achieve sustained virological response. Inversely, one patient (Case 8), with IL28B TT regardless of non-response at the first course, could achieve sustained virological response.

DISCUSSION

Mangia et al. [2010] reported that IL28B rs12979860 genotype was associated with sustained virological response to 24-week ribavirin combination therapy in HCV-2/3 patients who did not achieve rapid virological response, and that analysis of IL28B genotype might be used to guide treatment for these patients. In the present study of 24-week combination therapy in HCV-2 patients, IL28B rs8099917 TG + GG genotype was independent predictive factor for non-sustained virological response regardless of rapid virological response. The reasons of the discrepant results between the previous report and the present data are unclear, but these results suggest that treatment efficacy of HCV-2 to combination therapy might be predicted based on the combination of IL28B genotype and rapid virological response. Further prospective studies should be performed to develop the more effective treatment regimen with IL28B genotype, in HCV-2 patients.

Previous studies showed that IL28B rs8099917 genotype might affect treatment efficacy of 24-week ribavirin combination therapy in patients infected with HCV-2, and especially HCV-2b [Kawaoka et al., 2011; Sakamoto et al., 2011]. However, the present study for the whole population sample indicated that there were no significant differences in treatment efficacy, according to IL28B rs8099917 genotype. The discrepant results may be due to one or more factors. The first reason for this is probably the small number of patients in the present study (e.g., possible type error). The second reason is probably the difference of patients' background (lower age, and higher rates of past history of IFN monotherapy). The third reason is probably the difference of objects, based on the patients infected with HCV-2, who could complete 24-week combination therapy to minimize the influence of treatment regimen. Further studies of larger number of patients matched for background, including

TABLE V. Baseline Characteristics of HCV Genotype 2 Infected Patients at the Commencement of the Second Course Combination Therapy With Interferon Plus Ribavirin, and Treatment Efficacy at the First and Second Course of Combination Therapy

Case	Genotype	Sex	Age (years)	Albumin (g/dl)	ALT (IU/L)	HCV-RNA (log IU/ml)	*IL28B* rs8099917	First Tx (24 weeks)	Second Tx (48 weeks)
1	2b	Male	48	3.9	41	7.2	TT	Relapse	SVR
2	2b	Female	65	3.8	35	6.4	TT	Relapse	SVR
3	2b	Male	51	3.6	71	6.0	TT	Relapse	SVR
4	2a	Female	63	3.5	19	6.8	TT	Relapse	SVR
5	2a	Female	67	4.0	97	6.2	TT	Relapse	SVR
6	2b	Male	58	4.5	29	6.9	TT	Relapse	SVR
7	2b	Male	56	3.5	78	6.1	TG	Relapse	Relapse
3	2a	Male	57	3.6	240	6.7	TT	Non-response	SVR
9	2a	Male	40	3.8	434	5.8	TT	Non-response	Non-response*
10	2b	Female	55	3.5	132	6.1	TG	Non-response	Non-response*

SVR (sustained virological response): HCV-RNA undetectable at 24 weeks after the completion of therapy. Non-response: HCV-RNA detectable during or at the end of therapy. Relapse: HCV-RNA undetectable at the end of therapy, and detectable at 24 weeks after the completion of therapy. Tx: treatment.

*Two patients could not achieve a decrease in HCV-RNA of >2.0 log within 12 weeks after the commencement of treatment, so they were stopped combination therapy before the completion of 48-week therapy (12 weeks of case 9 and 22 weeks of case 10).

age, sex, genotype, past history of treatment, and treatment duration are required to investigate the association of IL28B genotype and viral response in patients infected with HCV-2.

In patients infected with HCV-1, previous studies have demonstrated that sustained virological response rates of late virological responders (HCV-RNA detectable at 12 weeks and undetectable at 24 weeks after the start of treatment) could be improved when treatment was extended to 72 weeks, compared with standard treatment duration of 48 weeks, largely as a result of reducing posttreatment relapse rates [Buti et al., 2003; Berg et al., 2006; Sánchez-Tapias et al., 2006; Pearlman et al., 2007; Akuta et al., 2009]. A pilot study of seven patients infected with HCV-2 showed that sustained virological response rates of patients, who were relapse at the first course of 24-week regimen, could be improved when treatment was extended to 48-week regimen [Akuta et al., 2010b]. However, the present study indicated that one patient (Case 7) could not achieve sustained virological response regardless of relapse at the first course of 24-week regimen, and that the other one (Case 8) could achieve sustained virological response regardless of non-response at the first course. The reason of the discrepant results might be due to IL28B genotype. In this study, all of six patients, with IL28B TT and relapse at the first course, could achieve sustained virological response, but two patients with IL28B TG could not achieve sustained virological response. To our knowledge, this is the first report to indicate that IL28B genotype and treatment efficacy at the first course of 24-week regimen might be important as pretreatment predictors of extending combination therapy for HCV-2. Furthermore, the more effective therapeutic regimens, including triple therapy of PEG-IFN plus ribavirin with telaprevir [Foster et al., 2011], should be developed for these patients, who could not achieve sustained virological response by extending dual therapy of IFN plus ribavirin. One limitation is that the present preliminary study was performed based on the small numbers of 10 patients with extending combination therapy for HCV-2. Further prospective studies of larger number of patients were required to investigate the pretreatment predictors of sustained virological response of extending combination therapy for HCV-2, including IL28B genotype and treatment efficacy at the first course of 24-week regimen.

Previous reports indicated that viral factors (e.g., viral load and periods from the start of treatment to initial point of undetectable HCV-RNA) and host factors (e.g., age, body mass index, and fibrosis stage) might be important predictors of treatment response to IFN plus ribavirin combination therapy in HCV-2, in addition to treatment-related factors (e.g., treatment duration, ribavirin dose, and prior treatment) [Mangia et al., 2005, 2009, 2010; Toyoda et al., 2009; Kawaoka et al., 2011; Sakamoto et al., 2011; Nagoshi et al., 2012]. In the present study, multivariate analysis identified these factors as predictors of sustained virological response. Recent report based on the meta-analysis indicated that insulin resistance (especially, HOMA-IR) might be also one of predictive factors for sustained virological response to combination therapy in HCV-2 [Eslam et al., 2011]. In the present study, the impact of glucose metabolism on treatment efficacy could not be evaluated, except for fasting plasma glucose. Further studies should be performed to investigate the clinical impact of insulin resistance on viral response of HCV-2.

In conclusion, the present results suggest that IL28B genotype might partly affect viral response of HCV-2 to IFN plus ribavirin combination therapy. The limitations of this study were that it could not investigate other races apart from Asians in Japan. Further prospective studies of larger number of patients matched for race and HCV genotype are required to explore the relationship between IL28B genotype and the response to combination therapy.

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Journal of Medical Virology 84:1199–1207 (2012)

Long-Term Interferon Monotherapy Reduces the Risk of HCV-Associated Hepatocellular Carcinoma

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The aims of this study were to evaluate the efficacy of long-term interferon (IFN) monotherapy on hepatocellular carcinoma (HCC) in patients who showed no virological response to the first course of IFN therapy, define predictive factors for HCC in patients on long-term IFN monotherapy, and evaluate the clinical impact of amino acid (aa) substitutions in the hepatitis C virus (HCV)-1b core region on HCC rate. This retrospective study included 494 consecutive treatment-naive patients infected with HCV-1b who failed to achieve sustained virological response after ≥24-week IFN monotherapy. Of 494 patients, 113 (22.9%) received another course of ≥48-week IFN monotherapy (additional-IFN group), while the remaining 381 (77.1%) received no such therapy (no-additional-IFN group), and 10 years have elapsed since the end of the first IFN monotherapy. The cumulative HCC rate was significantly higher in the no-additional-IFN group than additional-IFN group, and in those with aa substitutions in the core region of Gln70(His 70) and Met 91 than those with Arg 70 and/or Leu 91. Multivariate analysis identified stage of liver fibrosis, liver enzymes, age, treatment group, aa substitution in the core region, low-density lipoprotein cholesterol (LDL-cholesterol), and gender as determinants of HCC, and that additional IFN treatment significantly lowered the cumulative rate of HCC, even in patients with cirrhosis. In conclusion, long-term IFN monotherapy reduces the risk of HCC, even in patients with cirrhosis. Substitution of aa at position 70 and/or 91 in the core region and lipid metabolism are important predictors of HCC in long-term IFN monotherapy. ***J. Med. Virol. 84:1199–1207, 2012.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: HCV; genotype; interferon; HCC; core region, lipid metabolism

INTRODUCTION

Infection with hepatitis C virus (HCV) often progresses to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [Niederau et al., 1998; Kenny-Walsh, 1999]. At present, the combination of interferon (IFN) and ribavirin is the mainstay treatment of HCV infection. In Japan, 70% of HCV infections are caused by HCV genotype 1b (HCV-1b) and associated with high viral load, making treatment of patients with chronic hepatitis C often challenging and difficult [Tsubota et al., 2005].

Previous studies showed that IFN monotherapy reduces the risk of HCC [Nishiguchi et al., 1995; Ikeda et al., 1999; Yoshida et al., 1999; Arase et al., 2007; Nomura et al., 2007; McHutchison et al., 2008; Akuta et al., 2008]. Furthermore, a large scale cohort study has recently shown that patients with cirrhosis who were treated with IFN alone had a lower risk of HCC than those who did not during a median follow-up period of 6.7 years [Lok et al., 2011]. However, there are no reports of long-term follow up (more than 10 years) of IFN monotherapy, especially in patients who failed to achieve sustained virological response to IFN therapy, i.e., whether long-term IFN monotherapy reduces the risk of HCC on a long-term basis.

Despite numerous lines of epidemiological evidence of the association of HCV infection with HCC, it remains controversial whether the virus itself plays a direct or indirect role in the pathogenesis of HCC [Koike, 2005]. It has become evident that the HCV core region is potentially oncogenic in transgenic mice [Moriya et al., 1998], but the clinical impact of the core region on hepatocarcinogenesis is still unclear.

Grant sponsor: Ministry of Health, Labor and Welfare, Japan.

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Accepted 29 February 2012
DOI 10.1002/jmv.23288
Published online in Wiley Online Library (wileyonlinelibrary.com).

Whether substitutions of aa 70 and/or 91 in the HCV-1b core region affect hepatocarcinogenesis in patients who do not achieve sustained virological response to IFN therapy and then receive another course of long-term IFN monotherapy remains to be investigated.

The present study included 494 consecutive patients who were infected with HCV genotype 1b and failed to achieve sustained virological response after the first course of IFN monotherapy for more than 24 weeks. The aims of the study were the following: (1) To evaluate the long-term efficacy of additional long-term IFN monotherapy on HCC in patients who showed no virological response to the first IFN therapy. (2) To analyze the predictive factors for HCC in patients on long-term IFN monotherapy. (3) To evaluate the clinical impact of aa substitutions in the HCV-1b core region on HCC.

PATIENTS AND METHODS

Patients

Among 2,716 consecutive HCV-1b infected Japanese adult patients, in whom IFN monotherapy was induced between February 1987 and May 2006 at Toranomon Hospital, 494 were selected in this retrospective study based on the following criteria: (1) Patients naive to IFN, (2) patients negative for hepatitis B surface antigen (by radioimmunoassay, Dainabot, Tokyo), positive for anti-HCV (by a third-generation enzyme immunoassay, Chiron Corp., Emerville, CA) and for HCV RNA by qualitative or quantitative analysis, before IFN therapy, (3) patients infected with a single genotype of HCV-1b, (4) patients with chronic liver disease, without HCC before and during IFN therapy, (5) patients treated with IFN alone for more than 24 weeks, and showed no sustained virological response, (6) patients who did or did not receive additional ≥48-week IFN monotherapy, (7) patients who had not been treated with IFN plus RBV or PEG-IFN alone during follow-up, (8) patients free of coinfection with human immunodeficiency virus, (9) patients who have not been treated with antiviral or immunosuppressants within 6 months before enrolment, (10) lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake), and (11) patients free of other types of hepatitis, including hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease.

Figure 1 shows an overview of the study. All patients received the first treatment course of IFN alone (IFN-α and/or IFN-β) for more than 24 weeks, including initial aggressive induction therapy (every day within 8 weeks, followed by three times per week). Patients who did not achieve sustained virological response after the first course of IFN monotherapy were divided into two groups based on subsequent treatment with IFN alone; the additional-IFN group (representing patients who received another course of IFN monotherapy) and the no-additional-IFN group [representing patients who did not receive a subsequent course of IFN monotherapy based on concerns about adverse effects, lack of time for treatment, physician recommendation based on the appearance of depression, development of cardiopulmonary disease during or after the first course of IFN, and/or low levels of alanine aminotransferase (ALT)].

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital and a signed consent form was obtained from each patient. Table I summarizes the clinical features of 494 patients at the start of the first course of IFN monotherapy. They included 292 men and 202 women, aged 21–75 (median, 53 years). The numbers of patients with fibrosis of the liver stages 1/2 and 3/4 were 384 and 45 patients, respectively. The median follow-up period was 10.5 years (range, 0.0–18.0 years).

Laboratory Investigations

Blood samples were frozen at −80°C within 4 hr of collection and thawed before testing. HCV genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of the NS5 region [Chayama et al., 1993]. HCV RNA was quantitated by the branched DNA assay version 2.0 (Chiron Corp.), AMPLICOR GT HCV Monitor version 2.0 using the 10-fold dilution method (Roche Molecular Systems Inc., Pleasanton, CA), or COBAS TaqMan HCV test (Roche Diagnostics, Tokyo). A high viral load was defined as branched DNA assay ≥1.0 Meq/ml, AMPLICOR GT HCV Monitor ≥100 × 10^3 IU/ml, or COBAS TaqMan HCV test ≥5.0 log IU/ml. Low viral load was

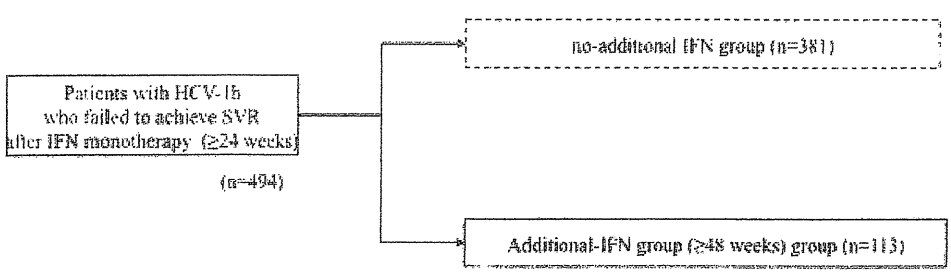

Fig. 1. Overview of the study.

TABLE I. Patient Characteristics at the Start of the First Course of IFN Treatment

Sex (male/female)	292/202
Age (year)	53 (21–75)
HCV genotype 1b	494
Fibrosis stage (F1/F2/F3/F4)	384/45
Aspartate aminotransferase (IU/l)	57 (18–348)
Alanine aminotransferase (IU/l)	84 (16–782)
Amino acid substitutions in core region of HCV genotype 1b [Gln70(His 70) and Met 91/Arg 70 and/or Leu 91)]	47/361
Treatment group (additional-IFN group)/ (no-additional-IFN group)	381/113
Total cholesterol (mg/dl)	166 (92–273)
Low-density lipoprotein cholesterol (mg/dl)	99 (28–280)
High-density lipoprotein cholesterol (mg/dl)	45 (18–102)
Triglyceride (mg/dl)	85 (28–437)
Body mass index (kg/m^2)	23 (16.7–35.2)

Data are number of patients or median values (range).

defined as branched DNA assay <1.0 Meq/ml, AMPLICOR GT HCV Monitor $<100 \times 10^3$ IU/ml, or COBAS TaqMan HCV test <5.0 log IU/ml. The lower limit of HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manaheim, Germany) was 100 copies/ml, and that of COBAS TaqMan HCV test was 1.2 log IU/ml. Samples with undetectable HCV RNA by qualitative analysis or COBAS TaqMan HCV test were defined as negative HCV RNA.

Detection of Amino Acid (aa) Substitutions in the Core Regions of HCV-1b

Basically, aa substitutions of the HCV-1b core region were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of antiviral therapy and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids of the core region were amplified by nested PCR using the following primers: The first-round PCR was performed with CE1 (sense, 5′-GTC TGC GGA ACC GGT GAG TA-3′, nucleotides: 134–153) and CE2 (antisense, 5′-GAC GTG GCG TCG TAT TGT CG-3', nucleotides: 1096–1115) primers, and the second-round PCR with CC9 (sense, 5′-ACT GCT AGC CGA GTA GTG TT-3′, nucleotides: 234–253) and CE6 (antisense, 5′-GGA GCA GTC GTT CGT GAC AT-3′, nucleotides: 934–953) primers. All samples were initially denatured at 95°C for 2 min. The 35 cycles of amplification were set as follow: Denaturation for 30 sec at 95°C, annealing of primers for 30 sec at 55°C, and extension for 1 min at 72°C with an additional 7 min for extension. Then, 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

With the use of HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on 50 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005].

Liver Histopathological Examination

Liver biopsy specimens from 429 patients were obtained percutaneously or at peritoneoscopy 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. All specimens for examination contained six or more portal areas. Histopathological diagnosis was made by an experienced liver pathologist (H.K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on histopathological assessment according to the scoring system of Desmet et al. [1994].

Diagnosis of Liver Cirrhosis

Cirrhosis was diagnosed based on the presence of markedly irregular surface with nodular formation in the liver, evident on peritoneoscopy, histological assessment according to the scoring system of Desmet et al. [1994], or on computed tomography (CT) or ultrasonography (US). Ascites, edema and esophageal varicosities, facilitated the diagnosis when present. Furthermore, 19 patients with cirrhosis underwent peritoneoscopy and/or liver biopsy; the remaining 18 patients did not undergo histological assessment, and therefore their diagnosis was established morphologically based on imaging examination.

Follow-Up and Diagnosis of Hepatocellular Carcinoma

Clinical and laboratory assessments were performed at least once every month before, during, and after treatment. Adverse effects were monitored clinically by careful interviews and medical examination at least once every month. Blood samples were also obtained at least once every month before, during, and after treatment. Patients were examined for HCC by abdominal US every 3–6 months. If HCC was suspected, additional procedures, such as magnetic resonance imaging, abdominal angiography, and US-guided tumor biopsy if necessary, were used to confirm the diagnosis. Follow-up time represented the time from the end of the first course of IFN treatment until death or until the last visit or until the start of

IFN-based treatment including IFN plus ribavirin therapy.

Statistical Analysis

Non-parametric tests were used to compare variables between groups, including the Mann–Whitney U-test. The cumulative rate of HCC was calculated using the Kaplan–Meier technique and differences between the HCC rate curves were tested using the log-rank test. Statistical analysis of the HCC rate according to groups was calculated using the period between the end of first IFN monotherapy and appearance of HCC. Stepwise Cox regression analysis was used to determine independent predictive factors associated with hepatocarcinogenesis. The hazard ratio (HR) and 95% confidence interval (95% CI) were also calculated. Potential predictive factors associated with hepatocarcinogenesis were sex, age, body mass index, aspartate aminotransferase (AST), ALT, total cholesterol, low-density lipoprotein cholesterol (LDL-cholesterol), high density lipoprotein cholesterol (HDL-cholesterol), triglyceride, treatment group, levels of viremia, stage of fibrosis, and aa substitutions in the core region. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into multivariate Cox proportional hazard model to identify significant independent factors that correlated with the HCC rate. Statistical comparisons were performed using the SPSS software (SPSS Inc., Chicago, IL). All P-values less than 0.05 by the two-tailed test were considered significant.

RESULTS

Background of Treatment Groups

Of the 494 patients who did not achieve sustained virological response after the first course of IFN of more than 24 weeks, 381 (77.1%) did not receive another course of IFN monotherapy (no-additional-IFN group), while the remaining 113 (22.9%) received another course of IFN monotherapy for more than 48 weeks (additional-IFN group); 24 patients of the latter group achieved sustained virological response following long-term IFN monotherapy.

Of the 494 patients, 418 (84.6%) received IFN-α alone; 55 patients (11.1%) received IFN-β alone; while the remaining 21 patients (4.3%) received a combination of IFN-α and IFN-β. In the no-additional-IFN group, the median duration of the first course of IFN was 24.9 weeks (range, 29–553.6 weeks). The median total dose of the first course of IFN was 630 MU (range, 16–7,873 MU). In the additional-IFN group, for the first and second courses of IFN monotherapy, the median duration of IFN was 24.4 weeks (range, 20–180.4 weeks) and 134.9 weeks (range, 63–752.4), respectively. In the additional-IFN group, for the first and second courses of IFN monotherapy, the median total dose of IFN was 624 MU (range, 210–2,777 MU) and 2,113 MU (range, 444–1,1373), respectively. There were no significant differences between the duration and total dose of the first course of IFN in the no-additional-IFN treatment group and those of the additional-IFN group (Mann–Whitney U-test). The median cumulative total duration and cumulative total dose for the additional-IFN group were 143.4 weeks (range, 23.7–867.7 weeks) and 2,756 MU (range, 1,002–12,020 MU), respectively.

Cumulative HCC Rates According to Treatment Group

During follow-up after the first course of IFN monotherapy, HCC was diagnosed in 62 (16.3%) patients of the no-additional-IFN group and 13 (11.5%) of the additional-IFN group, with cumulative HCC rates of 9.9, 5.2% at the end of 5 years; 18.7, 13.3% at the end of 10 years; and 34.6, 17.4% at the end of 15 years, for the no-additional-IFN and additional-IFN groups, respectively. The rates were significantly different between the two groups ($P = 0.035$; Log-rank test) (Fig. 2A).

HCC Rates According to aa Substitutions in the Core Region of HCV-1b

During follow-up after the first course of IFN monotherapy, 14 of 47 patients (29.8%) with Gln70(His 70) and Met 91, and 56 of 361 (15.5%) with Arg70 and/or Leu 91 developed HCC. In patients with Arg70 and/or Leu 91, the cumulative HCC rate was 4.7% at the end of 5 years; 16.4% at the end of 10 years; 27.9% at the end of 15 years. In patients with Gln70(His 70) and Met 91, the respective rates were 15.3, 27.3, and 46.6%. The rates for patients with Gln70(His 70) and Met 91 were significantly higher than those with Arg70 and/or Leu 91 ($P = 0.031$; Log-rank test) (Fig. 2B).

The subset data of 252 patients with Gln70(His 70) and/or Met 91 was also analyzed separately. HCC was diagnosed in 34 patients of the no-additional-IFN group and five patients in additional-IFN group, with cumulative HCC rates of 10.3, 3.8% at the end of 5 years; 22.9, 11.6% at the end of 10 years; 38.1, and 11.6% at the end of 15 years, for the no-additional-IFN and additional-IFN groups, respectively. The cumulative HCC rates were significantly different between the additional-IFN group and the no-additional-IFN group ($P = 0.022$; Log-rank test) (Fig. 2C).

Determinants of HCC by Multivariate Analysis

The entire data set was also analyzed to determine those factors that could predict HCC. Univariate analysis showed significant relationship between HCC and the following parameters: Stage of fibrosis of the liver (F3/4, $P < 0.001$), AST level (≥58 IU/L, $P < 0.001$), ALT level (≥100 IU/L, $P < 0.001$), age (≥55 years, $P = 0.001$), LDL-cholesterol level

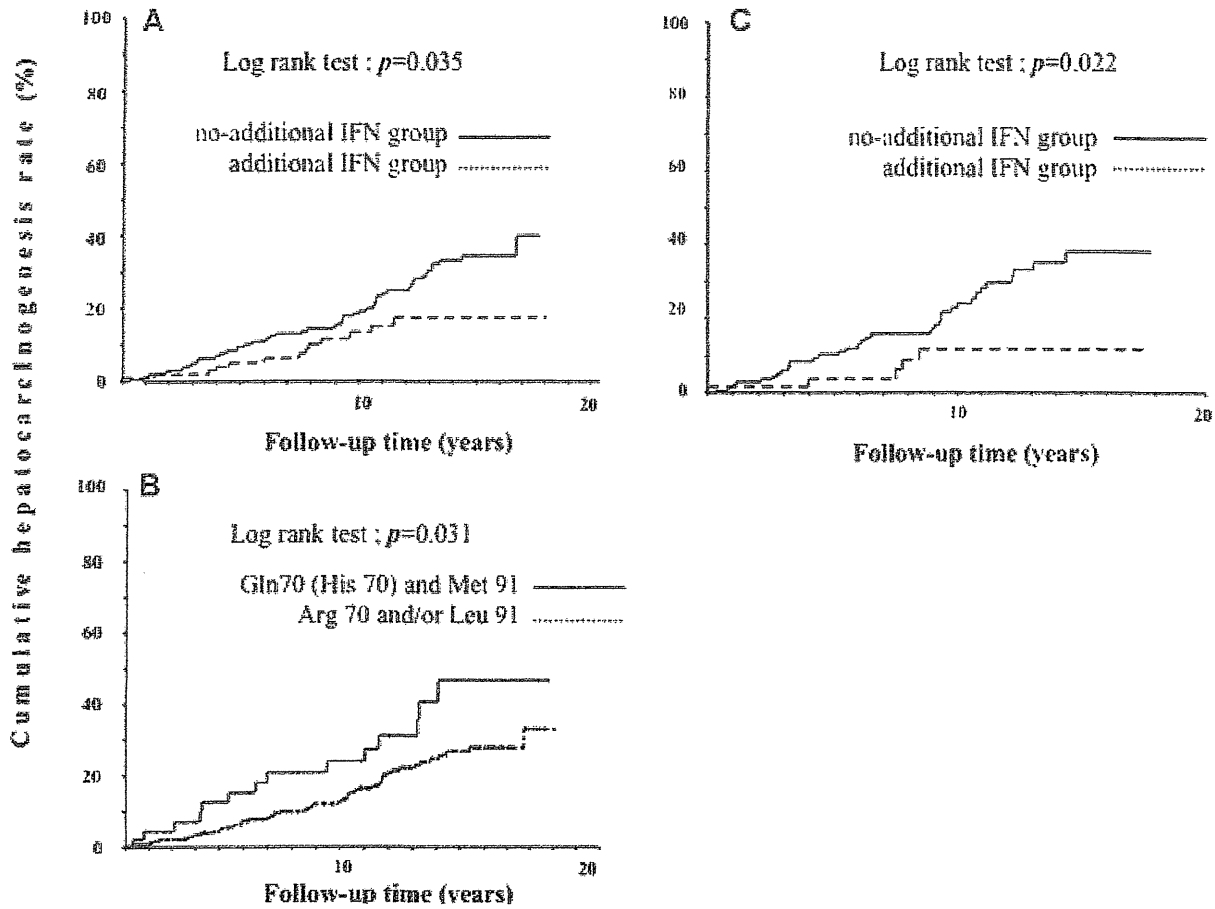

Fig. 2. Cumulative HCC rates according to study group (**A**), aa substitutions of core region (**B**), and patients with Gln70(His 70) and/or Met 91 substitutions (**C**). *P*-values by Log-rank test.

(<100 mg/dl, $P = 0.003$), sex (male, $P = 0.025$), aa substitutions in the core region (Gln70(His 70) and Met 91, $P = 0.031$), triglyceride level (≥100 IU/L, $P = 0.031$), and treatment group (no-additional-IFN group, $P = 0.035$). Next, these factors were entered into multivariate analysis, which then identified seven significant and independent determinants of HCC: Stage of fibrosis of the liver (F3/4; HR 9.98, $P < 0.001$), AST (≥58 IU/L; HR 3.27, $P = 0.001$), age (≥55 years; HR 2.71, $P = 0.002$), treatment group (no-additional-IFN group; HR 2.28, $P = 0.034$), substitution of aa 70 and/or 91 in the core region (Gln70(His 70) and Met 91); HR 2.21, $P = 0.024$), LDL-cholesterol (<100 mg/dl; HR 2.10, $P = 0.017$), and sex (male; HR 2.02, $P = 0.027$) (Table II).

Determinants of HCC in Patients With Cirrhosis by Multivariate Analysis

Data of the 37 patients with cirrhosis were also analyzed separately to determine those factors that could predict HCC in this subset of patients. Univariate analysis showed that sex (male) and IFN treatment tended to correlate ($P = 0.074$) and correlated significantly ($P = 0.032$) with HCC, respectively. Multivariate analysis identified treatment group (no-additional-IFN group. HR 3.04, $P = 0.040$) as the only independent parameter that significantly correlated with HCC (Table III).

DISCUSSION

Previous studies showed that IFN monotherapy can reduce the risk of hepatocarcinogenesis [Nishiguchi et al., 1995; Ikeda et al., 1999; Yoshida et al., 1999; Arase et al., 2007; Nomura et al., 2007; McHutchison et al., 2008; Akuta et al., 2008]. Furthermore, a recent large-scale cohort study showed that patients with cirrhosis who received IFN alone had a lower risk of HCC than those who did not receive such therapy after a median follow up of 6.7 years [Lok et al., 2011]. However, there is no report of follow up of IFN monotherapy for more than 10 years, especially in patients who had failed to achieve sustained virological