TABLE IIA. Factors Associated with Elevated Serum AFP Levels (≥11 μg/L) in Patients Infected with HCV Genotype 1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	*P*
Fibrosis stage	1: *F*1,2	1	
	2: *F*3,4	5.014 (2.746–9.153)	<0.001
Aspartate aminotransferase (IU/L)	1: <76	1	
	2: ≥76	4.592 (2.707–7.789)	<0.001
Substitution of aa 70	1: wild type	1	
	2: mutant type	2.618 (1.561–4.391)	<0.001
Platelet count ($\times 10^4/\mu l$)	1: ≥15.0	1	
	2: <15.0	1.912 (1.111–3.289)	0.019

*The presence of arginine at aa 70 was evaluated as wild type, while other patterns (glutamine/histidine) as mutant type. Normal reference ranges: ≤10 μg/L for alpha-fetoprotein.

The entire population sample was also analyzed to determine factors that could predict elevated AFP above twice ULN (≥21 μg/L); which was noted in 75 of 569 (13.2%) patients. Univariate analysis identified seven parameters that significantly influenced elevated AFP above twice ULN. These included age (≥45 years, $P = 0.015$), AST (≥76 IU/L, $P < 0.001$), ALT (≥100 IU/L, $P < 0.001$), platelet count ($<15.0 \times 10^4/\mu l$, $P < 0.001$), stage of fibrosis (*F*3,4, $P < 0.001$), and aa substitutions of the core region (mutant type of aa 70, $P < 0.001$, and aa 91, $P = 0.008$). Multivariate analysis identified four parameters that influenced independently elevated AFP above twice ULN, including stage of fibrosis (*F*3,4, $P < 0.001$), AST (≥76 IU/L, $P < 0.001$), and aa substitutions of the core region (HCV mutant-91, $P = 0.029$, and -70, $P = 0.056$) (Table IIB).

AFP Levels and aa Substitutions of Core Region

The entire population sample was also analyzed to determine the relationship between aa substitutions of the core region and AFP levels. The proportions of patients with HCV mutant-70 among those with AFP levels below 1, from 1 to 2, from 2 to 4, from 4 to 8, and above 8 times ULN were 33.4% (138 of 413 patients), 53.1% (43 of 81), 60.0% (24 of 40), 66.7% (8 of 12), and 69.6% (16 of 23) (Fig. 2A). Thus, the higher the proportion of patients with HCV mutant-70, the higher the AFP level, and significantly lower proportions of patients with HCV mutant-70 were noted among those with normal AFP levels (33.4%) than those with AFP levels from 1 to 2 times (53.1%) ($P = 0.001$) and above twice ULN (64.0%) ($P < 0.001$).

The proportions of patients with HCV mutant-91 among those with AFP levels below 1, from 1 to 2, from 2 to 4, from 4 to 8, and above 8 times ULN were 37.3% (154 of 413 patients), 40.7% (33 of 81), 67.5% (27 of 40), 25.0% (3 of 12), and 47.8% (11 of 23) (Fig. 2B). Thus, a higher frequency of HCV mutant-91 did not correlate with high AFP levels. In particular, significantly higher proportion of patients with HCV mutant-91 were noted among those with AFP levels from 2 to 4 times ULN (67.5%) than in those with AFP levels below 2 times (37.9%, $P < 0.001$) and above 4 times (40.0%, $P = 0.021$).

Normalization Rates of AFP Levels Based on Eradication of HCV-RNA With PEG-IFN Plus RBV Combination Therapy

Finally, the proportion of patients who showed normalization of AFP after commencement of PEG-IFNα-2b plus RBV combination therapy was determined in those at high risk for hepatocarcinogenesis, who had abnormal AFP levels (>10 IU/L) and HCV mutant-70 at baseline. Of the 340 patients, 49 had both abnormal AFP level and HCV mutant-70 at baseline. Of these, 14.3% (7 of 49 patients) could achieve sustained virological response, 28.6% (14 of 49) showed transient virological response, and 57.1% (28 of 49) had non-virological response. Table III summarizes the characteristics of

TABLE IIB. Factors Associated with Elevated Serum AFP Above Twice the Upper Limit of Normal (≥21 μg/L) in Patients Infected with HCV Genotype 1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	*P*
Fibrosis stage	1: *F*1,2	1	
	2: *F*3,4	6.875 (3.485–13.56)	<0.001
Aspartate aminotransferase (IU/L)	1: <76	1	
	2: ≥76	6.144 (3.088–12.23)	<0.001
Substitution of aa 91	1: wild type	1	
	2: mutant type	2.101 (1.077–4.099)	0.029
Substitution of aa 70	1: wild type	1	
	2: mutant type	1.914 (0.984–3.722)	0.056

*The presence of arginine at aa 70 was evaluated as wild type, and other patterns (glutamine/histidine) as mutant type. The presence of leucine at aa 91 was evaluated as wild type, and other pattern (methionine) as mutant type. Normal reference ranges: ≤10 μg/L for alpha-fetoprotein.

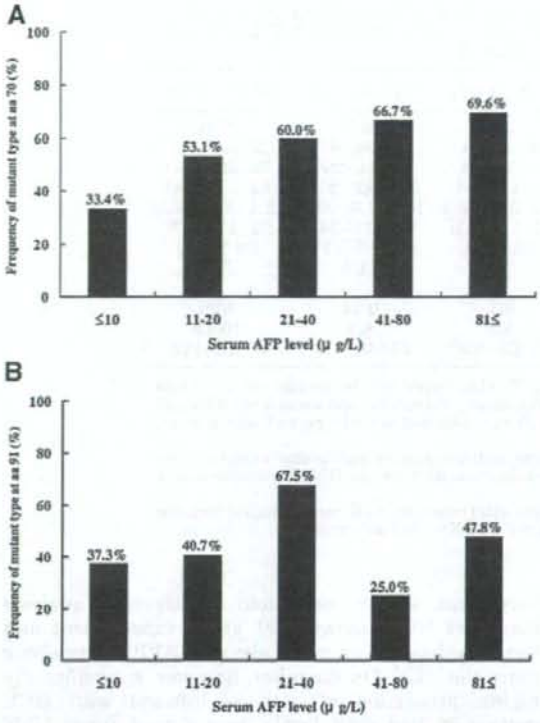

Fig. 2. A: Frequency of mutation in aa at position 70 of the HCV-1b core region according to serum AFP levels. Higher frequencies of the mutation correlated with higher serum AFP levels. Significantly lower frequencies of the mutant type were noted in patients with normal AFP levels (≤10 μg/L) than in those with levels from 1 to 2 times (11–20 μg/L, $P = 0.001$) and above twice the upper limit of normal (≥21 μg/L, $P < 0.001$), respectively. B: Frequency of mutation in aa at position 91 of the HCV-1b core region according to serum AFP levels. Higher frequencies of the mutation did not correlate with higher AFP levels. Significantly higher frequencies of the mutant type were noted in patients with AFP levels from 2 to 4 times the upper limit of normal (21–40 μg/L) than in those with levels below 2 times (≤20 μg/L, $P < 0.001$) and above 4 times (≥41 μg/L, $P = 0.021$).

these 49 patients at the commencement of combination therapy, according to treatment efficacy. The duration of treatment of non-virological responders was significantly shorter than that of sustained- ($P < 0.001$; Bonferroni test) and transient-virological responders ($P = 0.011$; Bonferroni test). Furthermore, AST levels of non-virological responders were significantly lower than those of sustained virological responders ($P = 0.049$; Bonferroni test). However, there were no significant differences in other patient characteristics at the commencement of treatment among the three groups.

The proportions of patients who showed normalization of AFP at the completion of treatment were 71.4% (5 of 7), 71.4% (10 of 14), and 53.6% (15 of 28) for the sustained-, transient-, and non-virological responders, respectively. There were no significant differences in the normalization rates at the completion of treatment among the three groups (Bonferroni test). However, the proportions of patients who showed normalization of AFP at 24 weeks after completion of treatment were 100% (7 of 7), 71.4% (10 of 14), and 28.6% (8 of 28) in the sustained-, transient-, and non-virological responders, respectively. The normalization rate in non-virological responders was significantly lower than in sustained- ($P = 0.001$; Bonferroni test) and transient virological responders ($P = 0.012$; Bonferroni test) (Fig. 3).

DISCUSSION

Elevated AFP in HCV-infected patients without HCC might be useful early predictor of hepatocarcinogenesis, but there is little evidence that mild elevation of AFP in such patients is associated with eventual development of HCC. Ikeda et al. [2006] reported that AFP level above twice ULN was an independent and significant determinant of hepatocarcinogenesis in patients with HCV-related cirrhosis. The present study of HCV-infected patients treated with IFN and followed for up to 15 years also showed that the rate of hepatocarcinogenesis was significantly higher in patients with abnormal AFP levels than in those with normal levels. In particular, the rate of hepatocarcinogenesis in patients with normal AFP levels was significantly lower than in those with levels above twice the ULN, and tended to be lower than in those with levels from 1 to 2 times ULN (i.e., mild elevation of AFP). To our knowledge, the present study is the first to report the hepatocarcinogenesis rates according to AFP levels in HCV-infected patients followed over a 15-year period, including mild elevation of AFP in patients without HCC.

Despite numerous epidemiologic studies linking HCV infection and the development of HCC, it remains controversial whether HCV itself plays direct or indirect role in the pathogenesis of HCC [Koike, 2005]. Studies using transgenic mice concluded that the HCV core region can potentially cause HCC [Moriya et al., 1998], but the clinical impact of HCV core region on hepatocarcinogenesis is still not clear. Previous studies identified substitutions in aa 70 and/or 91 in the HCV-1b core region and elevated AFP levels as predictors of poor virological response to PEG-IFN plus RBV [Akuta et al., 2005, 2006, 2007a,b,c; Donlin et al., 2007], and also as risk factors for hepatocarcinogenesis [Ikeda et al., 2006; Akuta et al., 2007d]. It is speculated that cases resistant to treatment might ultimately develop HCC. The present study indicated that mutation in aa 70 in the core region predicted elevation of AFP in HCV-infected non-HCC patients. These results support the oncogenic potential of the HCV core region and clinically link mutations in this region to HCC.

Previous reports identified PA28γ-dependent pathway as a mechanism of HCV-associated hepatocarcinogenesis. Moriishi et al. demonstrated that knockout of the PA28γ gene induced accumulation of HCV core protein in nuclei of hepatocytes of HCV core gene transgenic mice and disrupted the development of both hepatic steatosis and HCC [Moriishi et al., 2003, 2007]. Furthermore, HCV core protein also enhanced the

TABLE III. Patient Characteristics at Commencement of Combination Therapy of Pegylated Interferon α-2b Plus Ribavirin, of 49 Patients with Abnormal AFP Levels and Mutant Type of aa 70

	SVR (n = 7)	TVR (n = 14)	NVR (n = 28)
Sex (male/female)	3/4	9/5	12/16
Age (years)*	58 (43–64)	56 (34–63)	57 (43–66)
Serum aspartate aminotransferase (IU/L)*	83 (37–324)[a]	84 (34–266)	76 (28–135)
Serum alanine aminotransferase (IU/L)*	99 (41–344)	126 (42–504)	82 (37–218)
Platelet count ($\times 10^4/\mu l$)*	11.6 (8.0–19.3)	14.1 (7.5–20.6)	12.4 (6.6–27.3)
Serum alpha-fetoprotein (μg/L)*	17 (11–161)	21 (11–38)	22 (11–427)
Fibrosis stage (F1/F2/F3/F4/ND)	0/3/2/0/2	2/0/5/0/7	6/3/7/2/10
Level of viremia (high titer/low titer)**	7/0	14/0	27/1
Amino acid substitutions in core region***			
aa 70 (wild/mutant)	0/7	0/14	0/28
aa 91 (wild/mutant)	5/2	6/8	16/12
Treatment duration (weeks)	75 (60–85)[b]	53 (46–77)[c]	47 (12–112)

Data are number of patients, except those denoted by *, which represent the median (range) values. (**) Level of viremia was evaluated as high titer (≥1.0 Meq/ml, or ≥100 KIU/ml) and low titer (<1.0 Meq/ml, or <100 KIU/ml). (***) The presence of arginine at aa 70 was evaluated as wild type, and other patterns (glutamine/histidine) as mutant type.
The presence of leucine at aa 91 was evaluated as wild type, and other pattern (methionine) as mutant type.
Normal reference ranges: 11–38 IU/L for aspartate aminotransferase; 6–50 IU/L for alanine aminotransferase (IU/L); ≤10 μg/L for alpha-fetoprotein.
SVR: sustained virological response; TVR: transient virological response; NVR: non-virological response; ND: not done. [a]$P = 0.049$, [b]$P < 0.001$, [c]$P = 0.011$, compared with NVR by Bonferroni test.

binding of liver X receptor α (LXRα)/retinoid X receptor α (RXRα) to the LXR-response element in the presence of PA28γ [Moriishi et al., 2007]. Thus, PA28γ could play a crucial role in the development of HCV-associated steatogenesis and hepatocarcinogenesis. Further studies are necessary to link the results of animal studies and the clinical impact of aa substitutions in HCV core region on hepatocarcinogenesis.

Chu et al. [2001] indicated that elevation of AFP in the absence of HCC might be associated with HCV-1b infection, and that such rise could correlate with more severe hepatic necroinflammation and fibrosis/cirrhosis and higher viremia levels. The results of the present study indicated that patients infected with HCV mutation-70 had elevated serum AFP levels, although the relation between HCV mutation-91 and AFP was not very clear. On the one hand, multivariate analysis identified HCV mutation-91 as an independent and significant determinant of elevated AFP levels above twice the ULN. On the other; however, a significantly higher proportion of patients infected with HCV mutant-91 had AFP levels from 2 to 4 times ULN compared to those with levels below 2 times and levels above 4 times, i.e., there was no relation between the frequency of HCV mutant-91 and serum AFP levels. Further large-scale studies should be performed to investigate the relationship between HCV mutant-91 and elevated AFP.

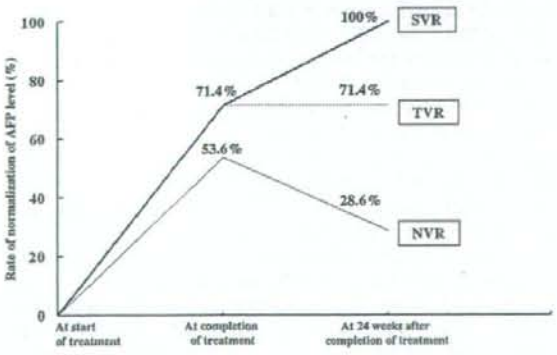

Fig. 3. Normalization rates of AFP levels at and 24 weeks after completion of treatment in sustained virological responders (SVR), transient virological responders (TVR), and non-virological responders (NVR).

Previous studies reported that IFN monotherapy [Arase et al., 2007] and IFN plus RBV combination therapy [Yu et al., 2006; Chen et al., 2007] results in reduction of AFP levels and the likelihood of hepatocarcinogenesis. In the present study, viral eradication (sustained virological response) in patients who received PEG-IFN plus RBV combination therapy was associated with normalization of AFP in patients at high risk for hepatocarcinogenesis (i.e., those with abnormal AFP levels and HCV mutant-70). These results emphasize that the risk of hepatocarcinogenesis could be reduced by eradication of HCV mutant-70. The results also showed that the proportion of patients with normalization of AFP levels was significantly higher in transient virological responders than in non-virological responders, suggesting that transient virological response could also result in the suppression of hepatocarcinogenesis, even when a sustained virological response is not achieved. In Japan, only 3 years had elapsed since the introduction of PEG-IFNα-2b plus RBV combination therapy into the Japanese Government Health Insurance system, and accordingly, the long-term effects of this combination therapy on hepatocarcinogenesis could not be evaluated in the present study. Further studies

that include patients treated with not only IFN but also PEG-IFN plus RBV, should be performed in the future.

In conclusion, the results of the present study indicated that substitution of aa at position 70 of the HCV-1b core region can predict elevation of serum AFP levels in non-HCC patients, and that eradication of the mutant virus seems to induce normalization of AFP. This finding highlights the importance of eradication of this mutant virus in reducing the risk of hepatocarcinogenesis. The limitations of the present study were that it did not investigate other genotypes apart from HCV-1b, the geographic diversities of HCV-1b core region (distribution of wild or mutant type), and the study of other races apart from Asians in Japan. Further prospective studies, matched for HCV genotype, aa substitutions of the core region, and race, of a large group of patients are required to determine the meaning of elevated AFP in non-HCC patients.

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Journal of Medical Virology 80:1363–1369 (2008)

Efficacy of Low-Dose Intermittent Interferon-Alpha Monotherapy in Patients Infected With Hepatitis C Virus Genotype 1b Who Were Predicted or Failed to Respond to Pegylated Interferon Plus Ribavirin Combination Therapy

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The efficacy of interferon (IFN) monotherapy for non-responders to pegylated interferon (PEG-IFN) plus ribavirin (RBV) combination therapy is still unclear. To evaluate the impact of IFN monotherapy on biochemical response, 200 consecutive patients infected with HCV genotype 1b, who received low-dose intermittent IFN-alpha monotherapy, were investigated. A median IFN dose per day of 3 million units was administered during a median period of 74 weeks. As a whole, the ALT normalization rates were 50.5, 65.9, 58.4, and 61.7% at 4, 12, 24, and 48 weeks, respectively. In 40 patients, who had abnormal AFP levels at the start of treatment, 52.5% achieved normalization of AFP within 48 weeks. Multivariate analysis identified indocyanine green retention rate at 15 min as the parameter that influenced significantly and independently ALT normalization. ALT normalization rates of patients who were predicted to be poor responders to PEG-IFN plus RBV combination therapy (but not substitutions of amino acid 70 and/or 91 in the HCV core region, female sex, and lower levels of low-density lipoprotein cholesterol) were similar to others. Furthermore, the ALT normalization rates in non-responders to combination therapy were 29.2, 60.9, 60.0, and 40.0% at 4, 12, 24, and 48 weeks, respectively. The results suggest that low-dose intermittent IFN monotherapy is an efficacious therapeutic regimen for patients unsuitable for PEG-IFN plus RBV, including non-responders, because it can lead to ALT normalization and thus a reduced risk of hepatocarcinogenesis. ***J. Med. Virol. 80:1363–1369, 2008.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: HCV; interferon; ribavirin; ALT; hepatocellular carcinoma; core region; AFP; low-density lipoprotein cholesterol

INTRODUCTION

Hepatitis C virus (HCV) usually causes chronic infection, which can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [Dusheiko, 1998; Ikeda et al., 1998; Niederau et al., 1998; Kenny-Walsh, 1999; Akuta et al., 2001]. Treatment of HCV-chronic hepatitis with interferon (IFN) can induce viral clearance and marked biochemical and histological improvement [Davis et al., 1989; Di Bisceglie et al., 1989].

Pegylated interferon (PEG-IFN) plus ribavirin (RBV) combination therapy for chronic HCV infection is expensive and associated with severe side effects but treated patients show a high-sustained virological response. Patients who do not achieve sustained virological response need to be identified before the start of combination therapy, in order to avoid unnecessary side effects and high costs. Thus, the safer IFN monotherapy should be selected as the therapeutic regimen for patients unsuitable for PEG-IFN plus RBV therapy. In a series of papers, Akuta et al. [2005a, 2006, 2007a,b,c] studied determinants of the response to PEG-IFN plus RBV in patients with high titers of genotype 1b (≥100 kiloIU [KIU]/ml), which is

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dominant in Japan. They identified substitutions of amino acid (aa) 70 and/or 91 in the HCV core region, female sex, and low levels of low-density lipoprotein cholesterol as independent and significant pretreatment negative predictors associated with virological response. Furthermore, previous studies reported that low-dose intermittent IFN monotherapy, as a treatment strategy, induces biochemical response [i.e., normalization of alanine aminotransferase (ALT) and alpha-fetoprotein (AFP) levels] and reduces the risk of hepatocarcinogenesis, even if patients failed to achieve sustained virological response [Arase et al., 2001, 2007; Nomura et al., 2007; McHutchison et al., 2008]. Hence, low-dose intermittent IFN monotherapy might be beneficial therapeutically in reducing the risk of hepatocarcinogenesis in patients who are predicted to be non-responsive to PEG-IFN plus RBV.

The present study included 200 consecutive patients infected with HCV genotype 1b, who were treated by self-injection of low-dose intermittent natural IFN-alpha. The aims of the study were the following. (1) To investigate the normalization rates of alanine aminotransferase (ALT) and α-fetoprotein (AFP) levels within 48 weeks after the commencement of treatment. (2) To examine the predictive factors associated with ALT normalization. (3) To evaluate the efficacy of IFN monotherapy in patients with predictors of poor response to IFN plus RBV combination therapy. (4) To evaluate the efficacy of IFN monotherapy for non-responders to IFN plus RBV combination therapy.

PATIENTS AND METHODS

Patients

Among 252 consecutive HCV-infected patients who started IFN monotherapy between April 2005 and July 2007 at Toranomon Hospital, 200 were selected in the present study based on the following criteria. (1) Patients treated by self-injection of natural IFN-alpha (Sumiferon®; Sumitomo Pharmaceutical Co., Osaka, Japan). (2) Patients infected with HCV genotype 1b alone. (3) Patients negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp, Emerville, CA), and positive for HCV RNA qualitative analysis with PCR (Amplicor, Roche Diagnostic Systems, Pleasanton, CA). (4) Patients who have not been treated with antiviral or immunosuppressive agents, except for IFN plus RBV combination therapy, within 6 months of enrolment. (5) Patients free of HCC. (6) Patients free of coinfection with human immunodeficiency virus. (7) Lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake). (8) Patients free of other types of hepatitis, including hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease, and (9) patients who consented to the study.

With regard to the clinical features of 200 patients at the start of IFN monotherapy, there were 103 men and 97 women, aged 27–77 with a median age of 62 years. The median ALT level was 80 IU/L (range, 6–487 IU/L), and the median platelet count was $13.0 \times 10^4/mm^3$ (range, 3.8×10^4–$28.0 \times 10^4/mm^3$). The median viremia level was 1,200 KIU/ml (range, 5–>5,000 KIU/ml) (Table I). Furthermore, 162 of the 200 patients (81%) received IFN-alpha monotherapy by three times per week; the remaining 38 patients (19%) received IFN-alpha monotherapy that included an initial daily administration in the first 8 weeks, followed by three times per week. A median IFN dose per day of 3 million units (MU, range; 3–6 MU) was administered during a median period of 74 weeks (range; 2–118 weeks). Of the 200 patients, 40 had not achieved sustained virological response with prior therapy of IFN plus RBV, and especially 27 patients of them had been treated with adequate combination therapy for at least 24 weeks (median, 43 weeks; range, 24–73 weeks).

Efficient treatment represented normalization of ALT levels (normal reference ranges: 6–50 IU/L) and AFP levels (normal reference ranges: ≤20 μg/L) during and at the end of 48-week treatment protocol.

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

Laboratory Investigations

Blood samples were obtained at least once every month from the commencement of treatment, and were tested for ALT and AFP levels. The serum samples were frozen at −80°C within 4 hr of collection and then 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 level was measured quantitatively by PCR (Cobas Amplicor HCV monitor v 2.0 using the 10-fold dilution method, Roche Diagnostics, Indianapolis, IN) at the commencement of treatment. The lower detection limit of the assay was 5 KIU/ml.

Detection of Amino Acid Substitutions in Core Region

With 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 genotype 1b was determined, and it was compared with the consensus sequence constructed on 50 clinical samples [Akuta et al., 2005a] for detecting substitutions at aa 70 of arginine (wild) or glutamine/histidine (mutant) and aa 91 of leucine (wild) or methionine (mutant). In the present study, aa substitutions of the core region were analyzed by direct sequencing [Akuta et al., 2005a, 2006]. The PCR genotyping could be performed in 193 patients; the remaining seven patients could not be analyzed due to the lack of adequate serum samples obtained before treatment.

Histopathological Examination of the Liver

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman

TABLE I. Patient Profile and Laboratory Data at Commencement of Interferon Monotherapy in 200 Patients Infected With HCV Genotype 1b

Demographic data	
Number of patients	200
Sex (M/F)	103/97
Age (years)*	62 (27–77)
History of blood transfusion	81 (40.5%)
Family history of liver disease	58 (29.0%)
Body mass index (kg/m^2)*	22.8 (15.6–32.9)
Laboratory data*	
Serum aspartate aminotransferase (IU/L)	69 (18–756)
Serum alanine aminotransferase (IU/L)	80 (6–487)
Serum albumin (g/dl)	3.7 (2.6–4.4)
Gamma-glutamyl transpeptidase (IU/L)	49 (11–368)
Leukocyte count ($/mm^3$)	4,000 (1,700–8,100)
Hemoglobin (g/dl)	13.9 (8.9–17.3)
Platelet count ($\times 10^4/mm^3$)	13.0 (3.8–28.0)
Indocyanine green retention rate at 15 min (%)	20 (4–62)
Serum iron (μg/dl)	146 (37–322)
Serum ferritin (μg/L)	136 (<10–1,308)
Creatinine clearance (ml/min)	99 (13–167)
Level of viremia (KIU/ml)	1,200 (5–>5,000)
Alpha-fetoprotein (μg/L)	9 (2–398)
Total cholesterol (mg/dl)	165 (15–296)
High-density lipoprotein cholesterol (mg/dl)	45 (21–80)
Low-density lipoprotein cholesterol (mg/dl)	96 (43–237)
Triglycerides (mg/dl)	93 (46–228)
Uric acid (mg/dl)	5.4 (2.8–9.4)
Fasting blood sugar (mg/dl)	97 (67–228)
Histological findings	
Stage of fibrosis (F1/F2/F3/F4/ND)	45/42/35/19/59
Hepatocyte steatosis (none to mild/moderate to severe/ND)	90/24/86
Treatment	
Interferon dose (million units/day)	3 (3–6)
Presence of initial daily interferon administration	38 (19.0%)
Amino acid substitutions in the core region[a]	
aa 70 (wild/non-wild/ND)	118/72/3
aa 91 (wild/non-wild/ND)	124/69/0
aa 70 and aa 91 (double wild/non-double wild/ND)	76/115/2

Data are number and percentage of patients, except those denoted by *, which represent the median (range) values.
Two patterns of mutant and competitive are indicated as non-wild. The pattern of wild at aa 70 and wild at aa 91 was evaluated as double wild-type, and the other patterns were non-double wild-type. ND, not determined.
[a]Amino acid substitutions were evaluated in 193 patient using pretreatment sera by direct sequencing.

needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), 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 (HK) 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].

Follow-Up

Clinical and laboratory assessments were performed at least once every month from the commencement of treatment. Adverse effects were monitored clinically by careful interviews and medical examination at least once every month. Patient compliance with treatment was evaluated with a questionnaire. Blood samples were also obtained at least once every month from the commencement of treatment, and were also analyzed for levels of ALT and AFP at various time points. Follow-up time represented the time from the start of treatment until the stop of treatment, or until the last visit.

Statistical Analysis

Analysis of efficacy of treatment was performed on an intention to treat basis. The χ^2 test, Fisher's exact probability test, and Mann–Whitney's *U*-test were used to compare the background characteristics between groups. The cumulative ALT normalization rates were calculated using the Kaplan–Meier technique; differences between the curves were tested using the log-rank test. Statistical analyses of ALT normalization according to groups were calculated using the period from the commencement of IFN monotherapy. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with ALT normalization within 48 weeks after the commencement of treatment. The odds ratios and 95% confidence intervals (95%CI) were also calculated. Potential predictive factors associated with ALT normalization

included the following 29 variables: sex, age, history of blood transfusion, family history of liver disease, body mass index, AST, ALT, albumin, γGTP, leukocyte count, hemoglobin, platelet count, indocyanine green retention rate at 15 min, serum iron, serum ferritin, creatinine clearance, level of viremia, AFP, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, fasting blood sugar, fibrosis stage, hepatocyte steatosis, IFN dose per day, and presence of initial daily IFN administration. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into a multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using the SPSS software (SPSS, Inc., Chicago, IL). All P values of less than 0.05 by the two-tailed test were considered significant.

RESULTS

Efficacy of IFN Monotherapy

The rates of ALT normalization in the intention to treat population analysis were evaluated at 0, 4, 12, 24, and 48 weeks after commencement of treatment. As a whole, the rates were 18.0% (36/200), 50.5% (94/186), 65.9% (116/176), 58.4% (90/154), and 61.7% (71/115), respectively. Thus, ALT normalization rates favorably exceeded 50% at 4 weeks. Furthermore, in 40 patients with abnormal AFP levels (≥21 μg/L) at the commencement of treatment, AFP levels of 92.5% (37/40) decreased and those of 7.5% (3/40) increased within 48 weeks. Especially, 52.5% (21/40) achieved normalization of AFP within 48 weeks. These results indicate that low-dose intermittent IFN monotherapy achieved favorable biochemical response.

Predictive Factors Associated With ALT Normalization by Univariate and Multivariate Analysis

The data for the whole population sample were analyzed to determine those factors that could predict ALT normalization within 48 weeks after the commencement of treatment. Univariate analysis identified nine parameters that tended to or significantly correlated with ALT normalization. These included AST ($P = 0.007$), ALT ($P = 0.009$), γGTP ($P = 0.075$), platelets ($P = 0.093$), fibrosis stage ($P = 0.066$), indocyanine green retention rate at 15 min ($P = 0.026$), serum iron ($P = 0.003$), high-density lipoprotein cholesterol ($P = 0.067$), and AFP ($P = 0.046$). These factors were entered into multivariate analysis, which then identified indocyanine green retention rate at 15 min ($P = 0.027$) as the parameter that influenced significantly and independently ALT normalization (Table II).

Efficacy of IFN Monotherapy in Patients With Predictors of Poor Response to PEG-IFN Plus RBV Combination Therapy

Figure 1 shows the prevalence with respect to ALT normalization rates in patients with predictors of poor response to PEG-IFN plus RBV combination therapy.

TABLE II. Factors Associated With ALT Normalization During Interferon Monotherapy, Identified by Univariate and Multivariate Analysis

		Univariate Cox proportional hazard model		Multivariate Cox proportional hazard model	
Factor	Category	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P
Aspartate aminotransferase (IU/L)	1:<70	1		—	—
	2:≥70	0.589 (0.400–0.867)	0.007	—	
Alanine aminotransferase (IU/L)	1:<75	1		—	—
	2:≥75	0.588 (0.395–0.875)	0.009	—	
γ-Glutamyl transpeptidase (IU/L)	1:<50	1		—	—
	2:≥50	0.636 (0.386–1.047)	0.075	—	
Platelets ($\times 10^4/mm^3$)	1:<15.0	1		—	—
	2:≥15.0	1.397 (0.946–2.064)	0.093	—	
Fibrosis stage	1:1.2	1		—	—
	2:3.4	0.627 (0.381–1.031)	0.066	—	
Indocyanine green retention rate at 15 min (%)	1:<20	1		1	—
	2:≥20	0.557 (0.333–0.932)	0.026	0.503 (0.274–0.925)	0.027
Serum iron (μg/dl)	1:<150	1		—	—
	2:≥150	0.522 (0.342–0.797)	0.003	—	
High-density lipoprotein cholesterol (mg/dl)	1:<45	1		—	—
	2:≥45	1.468 (0.973–2.215)	0.067	—	
Alpha-fetoprotein (μg/L)	1:<10	1		—	—
	2:≥10	0.662 (0.441–0.992)	0.046	—	

Only variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate and multivariate Cox proportional hazard model are shown.
95% CI, 95% confidence interval.

According to the substitutions of core aa 70 and aa 91, the ALT normalization rates were 15.7% (18/115) versus 22.4% (17/76) at 0 week, 42.6% (46/108) versus 61.4% (43/70) at 4 weeks, 62.1% (64/103) versus 69.7% (46/66) at 12 weeks, 59.3% (54/91) versus 56.1% (32/57) at 24 weeks, and 58.5% (38/65) versus 63% (29/46) at 48 weeks for non-double wild-type and double wild-type, respectively [not significantly different, except for 4 weeks ($P = 0.021$), Fig. 1A].

According to sex, the ALT normalization rates were 22.7% (22/97) versus 13.6% (14/103) at 0 week, 50.6% (44/87) versus 50.5% (50/99) at 4 weeks, 66.3% (53/80) versus 65.6% (63/96) at 12 weeks, 62.7% (42/67) versus 55.2% (48/87) at 24 weeks, and 67.3% (33/49) versus 57.6% (38/66) at 48 weeks for female and male, respectively (not significant, Fig. 1B).

According to the levels of low-density lipoprotein cholesterol, the ALT normalization rates were 15.5% (9/58) versus 20.0% (23/115) at 0 week, 37.0% (20/54) versus 54.1% (59/109) at 4 weeks, 58.8% (30/51) versus 66% (68/103) at 12 weeks, 58.5% (24/41) versus 53.8% (49/91) at 24 weeks, and 66.7% (22/33) versus 57.4% (39/68) at 48 weeks for low levels (<86 mg/dl) and high levels (≥86 mg/dl), respectively [not significant, except for 4 weeks ($P = 0.047$), Fig. 1C].

The above results indicate that in low-dose intermittent IFN monotherapy, ALT normalization rates of patients with predictors of poor response were not different from those without such predictors.

Efficacy of IFN Monotherapy in Non-Responders to IFN Plus RBV Combination Therapy

The rates of ALT normalization were evaluated in 27 patients who had received prior therapy with adequate IFN plus RBV for at least 24 weeks but showed no sustained virological response. The rates were 14.8% (4/27), 29.2% (7/24), 60.9% (14/23), 60% (12/20), and 40.0% (4/10) at 0, 4, 12, 24, and 48 weeks, respectively. Thus, ALT normalization rates favorably exceeded 60% at 12 weeks. These results indicate that non-responders to IFN plus RBV treated again with low-dose intermittent IFN monotherapy can achieve a favorable biochemical response.

DISCUSSION

For chronic HCV infection, PEG-IFN plus RBV combination therapy is expensive, associated with severe side effects but results in a high-sustained virological response. However, it is desirable to identify those patients who do not achieve sustained virological response before the start of the combination therapy in order to free them of unnecessary side effects and high costs. One alternative option for such patients is IFN

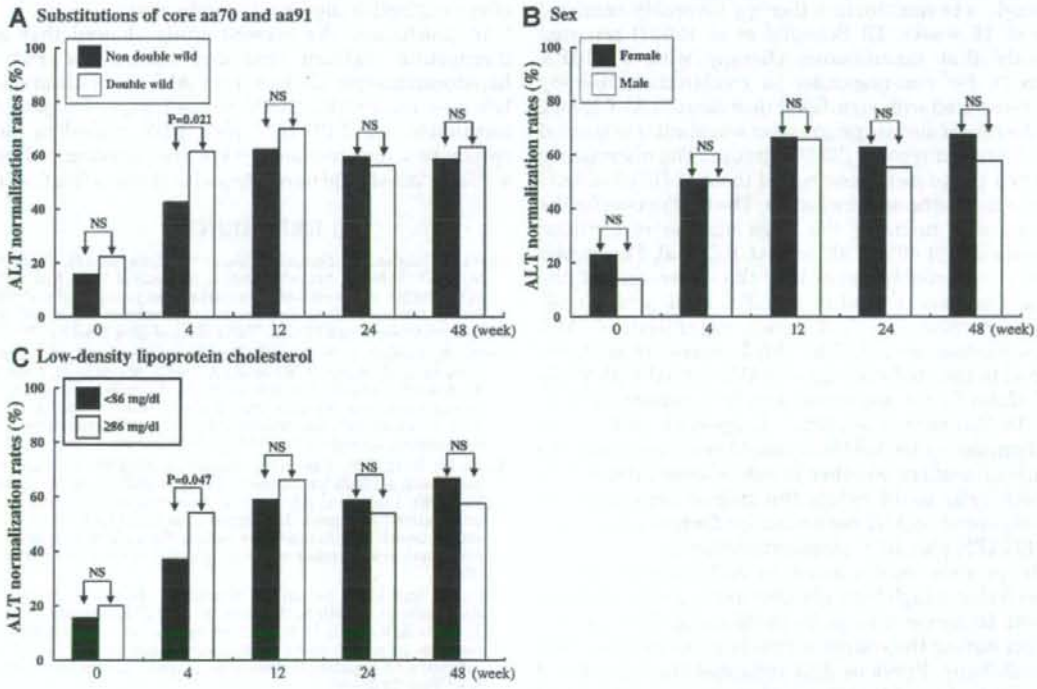

Fig. 1. The ALT normalization rates at 0, 4, 12, 24, and 48 weeks after commencement of IFN monotherapy, according to (**A**) substitutions of core aa 70 and aa 91, (**B**) sex, and (**C**) the levels of low-density lipoprotein cholesterol.

monotherapy, which could reduce the risk of hepatocarcinogenesis [Arase et al., 2001, 2007; Akuta et al., 2005a,b; Donlin et al., 2007; Nomura et al., 2007; McHutchison et al., 2008].

The efficacy of IFN monotherapy in patients who are predicted to respond poorly to IFN plus RBV combination therapy is still unknown. Previous results identified substitutions of aa 70 and/or 91 in the core region, female sex, and lower levels of low-density lipoprotein cholesterol as independent and significant pretreatment negative predictors associated with virological response [Akuta et al., 2005a, 2006, 2007a,b,c]. These studies also showed that substitutions of aa 70 and/or 91 are risk factors for hepatocarcinogenesis in the same patients [Akuta et al., 2007d]. The present study of low-dose intermittent IFN monotherapy showed that ALT normalization rates of patients who were predicted to have a poor response to the combination therapy were not different from others. It is important to achieve a better biochemical response regardless of core aa substitutions as risk factor of hepatocarcinogenesis. Thus, a low-dose intermittent IFN monotherapy for patients predicted to fail to respond to PEG-IFN plus RBV is an efficacious therapeutic regimen for normalization of ALT and thus reduction of risk of hepatocarcinogenesis.

The efficacy of IFN monotherapy for non-responders to IFN plus RBV combination therapy is still controversial. In the present study of low-dose intermittent IFN monotherapy, ALT normalization rates in non-responders to combination therapy favorably exceeded 60% at 12 weeks. Di Bisceglie et al. [2007] reported recently that maintenance therapy with PEG-IFN alpha-2a for non-responder to combination therapy was associated with significant decreases in ALT levels, but the rate of disease progression was similar in treated (34.1%) and untreated (33.8%) groups. The discrepancy between the present results and those of HALT-C trial may be due to one or more factors. The first reason for the difference is probably the large number of cirrhotic patients (about 40% of all) in HALT-C trial. The second reason is probably related to the difference in the efficacy measures used in HALT-C trial, which evaluated decreases in ALT levels regardless of ALT normalization or not. The third reason is probably related to the study design of HALT-C trial with PEG-IFN alpha-2a for non-responders to combination therapy [Di Bisceglie et al., 2007]. Large-scale prospective randomized controlled trials should be conducted in the future to confirm whether low-dose intermittent IFN monotherapy could reduce the risk of hepatocarcinogenesis based on ALT normalization for non-responders to PEG-IFN plus RBV combination therapy.

The present results based on multivariate analysis showed that a high level of indocyanine green retention rate at 15 min is a negative predictor of ALT normalization during the course of low-dose intermittent IFN monotherapy. Previous data indicated that absence of advanced liver fibrosis is a positive predictor of sustained virological response to IFN monotherapy and IFN plus RBV combination therapy [Jouet et al., 1994; Poynard et al., 2000; Bruno et al., 2004]. Akuta et al. [2005a, 2007a] also showed previously that high levels of indocyanine green retention rate at 15 min or low levels of serum albumin are associated with advanced liver fibrosis, and that they are independent and significant negative predictors of the virological response to PEG-IFN plus RBV. To our knowledge, this is the first report that describes the relationship between indocyanine green retention rate at 15 min level and biochemical response during IFN monotherapy. Di Bisceglie et al. [2007] recently reported that maintenance therapy with PEG-IFN alpha-2a failed to halt liver disease progression in patients with advanced hepatic fibrosis. Further studies of large number of patients are required to investigate the relationship between severity of histopathological liver changes and biochemical response during low-dose intermittent IFN monotherapy.

One limitation of the present study was that viral factors other than the core region of HCV genome were not examined, such as the interferon sensitivity determining region (ISDR) and the interferon/ribavirin resistance determining region (IRRDR) of NS5A region [Enomoto et al., 1995, 1996; El-Shamy et al., 2007]. Biochemical response during low-dose intermittent IFN monotherapy seems to be based on a dynamic tripartite interaction of virus, host, and treatment regimen. Further understanding of the complex interaction between these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, the present study showed that one therapeutic regimen that can reduce the risk of hepatocarcinogenesis based on ALT normalization is low-dose intermittent IFN monotherapy, for patients unsuitable for PEG-IFN plus RBV including non-responders. Large-scale prospective randomized controlled trials should be conducted to confirm this finding.

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Efficacy in Patients with Dose Reduction in Combination Therapy of Peginterferon and Ribavirin for Chronic Hepatitis C

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Key Words
Chronic hepatitis C · Peginterferon · Ribavirin · Dose reduction

Abstract

Objective: The aim of this study was to elucidate efficacy after dose reduction in combination therapy of peginterferon and ribavirin for chronic hepatitis C. ***Methods:*** Inclusion criteria were hepatitis C virus (HCV) genotype 1b, serum HCV RNA level of ≥100 KIU/ml, dose reduction of peginterferon and/or ribavirin between the first 4 weeks and 20 weeks after the initiation of treatment. 164 patients were enrolled in this retrospective cohort study. Predictive factors for sustained viral response (SVR) after dose reduction were examined. ***Results:*** Out of the 146 patients treated with dose reduction, 57 had SVR. Multivariate analysis showed that SVR occurred when serum HCV RNA at the time of dose reduction was negative ($p < 0.001$) and total ribavirin dose was ≥100% of the anticipated total dose ($p < 0.001$). 57% (55/97) of patients with undetectable serum HCV RNA at the time of dose reduction had SVR. In contrast, only 4% (2/49) of patients with detectable serum HCV RNA at the time of dose reduction had SVR. ***Conclusions:*** On dose reduction of combination therapy for chronic hepatitis C, undetectable serum HCV RNA at the time of dose reduction and attainment of the total ribavirin dose of ≥100% enhance SVR.

Introduction

Combination therapy of peginterferon and ribavirin for hepatitis C virus (HCV) induces sustained virological response (SVR) in 50–60% of cases with genotype 1 and in 80–90% of cases with genotype 2. These SVR rates in patients treated with combination of peginterferon and ribavirin were higher than those treated with interferon (IFN) alone [1–6]. Thus, combination therapy of peginterferon and ribavirin has been recommended as a first choice for chronic hepatitis C. However, combination therapy has been associated with various adverse events, such as psychological disturbances, poor appetite, skin rash, infection, anemia and leukopenia [1, 2, 5, 7]. Dose reduction or even discontinuation of treatment often becomes necessary in combination therapy for chronic hepatitis C. In several studies, the reduction rate due to severe side effects was reported to be about 25–40%.

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Some authors have reported that adherence to combination therapy enhanced sustained virological eradication in genotype 1 with chronic hepatitis C [8–12]. In the present study, we evaluated the efficacy after dose reduction of combination therapy in Japanese patients. The study design is non-randomized retrospective cohort study.

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: (1) HCV genotype 1b; (2) serum level of HCV RNA of ≥100 KIU/ml; (3) dose reduction of peginterferon and ribavirin between the first 4 weeks and 20 weeks after the initiation of combination therapy; (4) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; (5) no hepatitis B surface antigens, antinuclear antibodies, or antimitochondrial antibodies detectable in serum, determined by radioimmunoassay, and (6) leukocytes >2,000/mm^3, platelet count >80,000/mm^3, and bilirubin <2.0 mg/ml. We excluded from the study all the patients with the following: (1) a history of alcohol abuse; (2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The Institutional Ethics Review Board of our hospital approved our study. The physician in charge explained the purpose and method of this clinical trial, as well as the potential adverse reactions, to each patient, who later gave his/her informed consent for participation.

Combination Therapy of Pegylated-IFN and Ribavirin

For the treatment regimen, the peginterferon (Peg-intron; Schering-Plough Pharmaceutical Co., Osaka, Japan) and ribavirin (Rebetol; Schering-Plough Pharmaceutical Co.) were given based on body weight. At the initiation of combination therapy, patients received peginterferon at a median dose of 1.5 μg/kg (range 1.2–1.6 μg/kg) subcutaneously each week and oral ribavirin at a median dose of 11.9 mg/kg (range 10.0–16.3 mg/kg) daily. The peginterferon dose was adjusted according to body weight (60 μg for ≤40 kg, 80 μg for >40 and ≤60 kg, 100 μg for >60 and ≤80 kg, and 120 μg for >80 and ≤100 kg). The ribavirin dose was adjusted according to body weight (600 mg for ≤60 kg, 800 mg for >60 and ≤80 kg, and 1,000 mg for >80 kg).

The physician in charge reduced the dose of treatment when the blood cell count decreased. Peginterferon was stepwise reduced 20 μg/week if the WBC declined to <1,500/mm^3, leukocyte count to <750/mm^3 or platelet count to <80,000/mm^3. Ribavirin was stepwise reduced 200 mg if the hemoglobin level declined to ≤10 g/dl. The doses of peginterferon and ribavirin could be increased back to starting doses if these adverse events resolved. The amounts of both medications taken by each patient were expressed as a percentage of the anticipated total dose in the 48-week treatment regimen based on body weight. The median duration of treatment was 48 weeks (range 8–96 weeks). A SVR to therapy was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV, Version 2.0; Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy [13].

Blood samples were obtained just before and 6 months after combination therapy. The samples were stored at –80° until analyzed. Using these blood samples, the HCV RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0; Roche Molecular Systems) [14]. HCV genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously [15]. The start of the follow-up period was defined as the first day of dose reduction in combination therapy. Clinical evaluation and biochemical and hematological tests were performed at 1- to 4-weekly intervals. We evaluated the following: (1) SVR after dose reduction, and (2) predictive factors for SVR after reduction based on combination therapy-related side effects.

Liver Histology before IFN Therapy

Liver biopsy specimens were obtained percutaneously under the observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The biopsy specimens were scored according to the system of Desmet et al. [16].

Statistical Analysis

A Cox proportional hazards model was used to analyze the factors contributing to the stop of treatment and dose reduction due to combination therapy: factors examined included age, sex, body mass index, histological findings, HCV load, ALT, hemoglobin, WBC, platelet count, HCV RNA at the time of dose reduction, total ribavirin dose and peginterferon dose. Significance of trends in values was determined with a Cochran-Armitage Trend Test. $p < 0.05$ was considered statistically significant. The SPSS software package (SPSS 11.0 for Windows; SPSS Inc., Chicago, Ill., USA) was used for analyses.

Results

Clinical Characteristics of the Patients

A total of 146 patients were enrolled in the present study. The clinical characteristics of patients before combination therapy are shown in table 1. In 15 patients, a liver biopsy was not available because the patients declined to have a biopsy taken. Reduction time after the initiation of combination therapy was 12.0 ± 12.7 weeks (mean ± SD).

Predictors for SVR after Dose Reduction in Combination Therapy

Out of the 146 patients treated with dose reduction, 57 had SVR. Univariate analysis showed that the following seven factors significantly affected the SVR rate in all the patients: HCV RNA at the time of dose reduction ($p < 0.001$), HCV RNA at week 12 ($p < 0.001$), peginterferon

Table 1. Clinical characteristics before combination therapy of peginterferon and ribavirin in chronic hepatitis C patients (n = 146)

Characteristics	Patients, n or median (range)
Age, years	55 (20–69)
Male/female	81/65
Body weight, kg/height, cm	64.9 (36.7–96.6)/ 163.2 (135.2–185.5)
Body mass index	23.1 (16.6–32.0)
History of interferon therapy (–/+)	79/67
Liver histology (fibrosis, mild/moderate/severe)	61/47/23
HCV load, KIU/ml	1,500 (105–5,000)
AST/ALT, IU/l	55 (20–324)/76 (13–580)
Hemoglobin, g/dl	14.4 (10.4–17.9)
Platelets, $\times 10^4/mm^3$	14.1 (8.4–26.0)
WBC, $\times 10^3/mm^3$	4,200 (2,000–8,800)

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; WBC = white blood cells.

and/or ribavirin dose ($p < 0.001$), sex ($p < 0.001$), HCV RNA level before treatment ($p = 0.024$), histopathological staging ($p = 0.031$), and HCV RNA at week 24 ($p = 0.042$) (table 2A). The variables were mutually correlated and multivariate Cox regression analysis was performed with the seven statistically significant variables in the model. As shown in table 2B, multivariate analysis showed that SVR occurred when serum HCV RNA at the time of dose reduction after the initiation of combination therapy was negative ($p < 0.001$) and total ribavirin dose was ≥100% ($p < 0.001$).

SVR Rate Based on Adherence of Combination Therapy in Patients with Dose Reduction

The SVR rate based on adherence of combination therapy in patients with dose reduction was evaluated. Patients were divided into two groups based on the negativity or positivity of HCV RNA at the time of dose reduction after the initiation of combination therapy. Table 3A shows the SVR rate based on adherence of combination

Table 2. Predictive factors for SVR after dose reduction, based on combination therapy-related side effects

A Univariate analysis

Factor	Category	Odds ratio	95% CI	p value
HCV RNA at the time of reduction	–/+	1/0.09	0.04–0.21	<0.001
HCV RNA at week 12	–/+	1/0.09	0.04–0.22	<0.001
Total ribavirin dose, %	<100/≥100	1/8.58	3.99–18.47	<0.001
Total peginterferon dose, %	<100/≥100	1/3.37	1.672–7.04	<0.001
Sex	male/female	1/0.20	0.10–0.42	<0.001
HCV RNA, MEq/ml	<1,000/≥1,000	1/0.43	0.21–0.90	0.024
Liver histology, fibrosis	mild/moderate or severe	1/0.46	0.23–0.93	0.031
HCV RNA at week 24	–/+	1/0.12	0.01–0.92	0.042
Hemoglobin, g/dl	<13/≥13	1/2.29	0.87–5.98	0.092
Platelets, $\times 10^4/mm^3$	<10/≥10	1/2.55	0.86–7.61	0.092
WBC, mm^3	<3,000/≥3,000	1/1.14	0.40–1.27	0.062
ALT, IU/l	≥100/<100	1/1.31	0.59–2.93	0.164
Body mass index	<25/≥25	1/1.24	0.59–2.62	0.566
Age, years	<55/≥55	1/1.13	0.35–3.69	0.838

B Multivariate analysis

Factor	Category	Odds ratio	95% CI	p value
HCV RNA at the time of reduction	–/+	1/0.10	0.04–0.28	<0.001
Total ribavirin dose, %	<100/≥100	1/6.90	2.54–19.05	<0.001

ALT = Alanine aminotransferase; WBC = white blood cells.

Table 3. SVR rate based on adherence of combination therapy in patients with **A** negativity and **B** positivity of serum HCV RNA at the time of dose reduction[1]

A

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

B

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

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

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

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

Reasons for Dose Reduction

Of 146 patients with dose reduction, 40 had dose reduction of peginterferon only. 53 patients had dose reduction of ribavirin and 53 patients had both reduction of peginterferon and ribavirin. The cause of reduction accounted for the following: anemia 87 (59.6%), leukopenia 39 (26.7%), thrombocytopenia 22 (15.1%) and other reason, such as general fatigue, 46 (31.5%). 55 of 146 patients with dose reduction had two causes for dose reduction.

Discussion

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

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

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

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

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

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

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

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

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Abstract

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

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

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

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

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

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Introduction

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

On the other hand, several predictive factors of sustained viral response (SVR) to IFN have been identified, and these include short duration of disease, young age, absence of liver cirrhosis, genotype 2a, low hepatitis C virus (HCV)-RNA levels, HCV and mutant type of nonstructual5A region (9-15). Patients with liver cirrhosis (LC) have a high development of hepatocellular carcinoma (HCC) and progression to decompensated state. Thus, patients with a cirrhotic state should be treated for protection of progression of LC stage. In particular, LC patients with genotype 2a and low HCV-RNA levels might have the possibility of eradication of HCV RNA with a small dose or a short period of interferon (IFN). However, there is also controversy over how long the IFN therapy should be continued to eradicate HCV RNA in

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

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

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

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

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

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

Materials and Methods

Patients

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

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

Blood testing

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

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