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Prolonged-Interferon Therapy Reduces Hepatocarcinogenesis in Aged-Patients With Chronic Hepatitis C

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The aim of this study was to elucidate the reduction of hepatocarcinogenesis by prolonged interferon (IFN) monotherapy in aged chronic hepatitis C patients. Inclusion criteria were biopsy-proven chronic hepatitis or liver cirrhosis, 60 years and over, elevated serum aminotransferase and positive hepatitis C virus (HCV)-RNA. One hundred and twenty patients satisfied the above criteria were treated with natural IFN-alpha (dose: 3 million unit (MU), two or three times weekly for 0.5–15.5 years, mean 2.47 years) (IFN group). Another 240 patients treated with herbal medicines excluding IFN were selected as control (no-IFN group). The patients not treated with IFN were matched 2:1 with IFN group patients for sex and age. The clinical and biological differences were compared after treatment with the IFN group and the untreated group. Serum alpha-fetoprotein (AFP) level decreased with statistical significance after initiation of treatment with IFN compared to no treatment. The 5- and 10-year cumulative rates of hepatocellular carcinoma (HCC) were 5.9 and 13.7%, and 17.1 and 32.8%, for the IFN and untreated group, respectively. HCC development occurred when histologic staging was advanced, and IFN was not given, the AFP level after treatment was >10 ng/ml. Cox regression analysis indicated that the relative risk of HCC in patients in the IFN group was 0.3 times of that in the untreated patients. The relative risk rate for HCC in severe fibrosis was 3.9 compared with mild or moderate fibrosis. In conclusion, long-term IFN therapy for aged patients with chronic HCV infection is effective in decreasing the serum AFP level and preventing hepatocarcinogenesis. ***J. Med. Virol. 79:1095–1102, 2007.***

KEY WORDS: chronic hepatitis C; hepatocellular carcinoma; long-term interferon treatment; alpha-fetoprotein

INTRODUCTION

Current interferon (IFN) therapy for patients with chronic hepatitis C viral infection has been directed at viral clearance. Recent studies described improvement of therapeutic efficacy when IFN was combined with ribavirin [Schalm et al., 1997; McHutchison et al., 1998; Poynard et al., 1998; Reichard et al., 1998]. Novel long-acting formulations of IFN known as pegylated IFN induced a higher eradication rate of hepatitis C virus (HCV) [Zuzem et al., 2000; Lindsay et al., 2001; Manns et al., 2001]. However, some patients do not clear the virus despite these new IFN therapies. Failure of HCV clearance could lead potentially to liver cirrhosis and/or hepatocellular carcinoma (HCC) [Imai et al., 1998; Yoshida et al., 1999]. Some patients cannot be given full doses of IFN because of IFN-related side effects. Thus, it is necessary to develop a new strategy for preventing the development of HCC in patients who cannot clear HCV-RNA regardless of IFN therapy and cannot be given full doses of IFN because of related side effects.

IFN can prevent the development of some malignancies apart from eradicating HCV [Gutterman, 1994;

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon.

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Fogler et al., 1994; Scarpa et al., 1997; Murphy et al., 2001; Lindner, 2002]. A few previous studies indicate that long-term IFN therapy reduces the development of HCC in patients with HCV and chronic hepatitis or cirrhosis [Nishiguchi et al., 1995; Ikeda et al., 2000]. However, there is also controversy as to whether patients should be treated to prevent the development of HCC.

Some patients in Japan with chronic hepatitis C were, generally, aged. Also, HCV-related HCC patients have been shown to become old with a peak around age 70. Thus, this match-controlled study was conducted to evaluate the effect of long-term IFN therapy on the development of HCC in aged patients with HCV and with chronic hepatitis or cirrhosis.

MATERIALS AND METHODS

Patients

The number of patients who were diagnosed with chronic HCV infection and were subsequently treated with IFN monotherapy or IFN and ribavirin combination therapy between April 1991 and March 2006 was 4,250. Seven hundred and twenty of these patients had the following criteria: (1) laparoscopy and liver biopsy which showed histopathological features of chronic hepatitis or cirrhosis was taken within 1 year of initiation of IFN therapy; (2) 60 years and over; (3) positive for HCV-RNA by the amplicor monitor assay [Albadalejo et al., 1998] or reverse transcription nested polymerase chain reaction (RT-nested PCR) [Hagiwara et al., 1992]; (4) average alanine aminotransferase (ALT) elevation greater than 1.5 times the upper normal limits (ALT normal range: 12–50 IU) for more than 6 months before IFN therapy; (5) no treatment with corticosteroids, immunosuppressive agents, or antiviral agents within 12 months; (6) negative for hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) in the serum, as determined by radioimmunoassay and spot hybridization; (7) Leukocytes $>2{,}500/mm^3$, platelets $>70{,}000/mm^3$, and bilirubin <2.0 mg/ml before the initial period of IFN therapy; and (8) no evidence of HCC nodules by ultrasonography and/or computerized tomography within 1 month before IFN therapy.

Of the 720 patients satisfied with above criteria, 120 received IFN to prevent the development of HCC at a dose of 3 million units (MU) of natural IFN-alpha (Sumitomo Pharmaceutical Co., Osaka, Japan) two or three times a week for 2.47 ± 2.65 years. The decision for IFN therapy was made mainly after discussion between physician and patient. The patients were prospectively monitored the serum aminotransferase, alpha-fetoprotein (AFP), and HCC development. On the other hands, out of 720 patients with the above criteria, 240 patients (no-IFN group) treated without IFN were selected retrospectively so that no-IFN group patients were matched 2:1 with IFN group patients for sex, ages, and severe fibrosis. Patients with either of the following criteria were excluded from the study: (1) AFP of 400 ng/ml or higher, (2) advanced and decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites, (3) a short follow-up period of 6 months or less, or (4) IFN was given daily, at a dose of >6 MU or other IFN excluded natural IFN-alpha. We compared the clinical and biological differences between IFN group and untreated group. The patients not treated with IFN were given herbal medicines (e.g., vitamin K, ursodeoxycholic acid, glycyrrhizin) [Takano et al., 1994; Arase et al., 1997; Tsubota et al., 1999]. Some of these substances improve serum transaminase and/or protecting HCC appearance [Takano et al., 1994; Arase et al., 1997; Tsubota et al., 1999]. Therefore, these drugs have been used for chronic hepatitis or cirrhosis in Japan since 1979. Untreated patient did not receive corticosteroids, immunosuppressive agents, or antiviral agents during the first stage of treatment. The study was approved by the institutional ethics review. Each patient gave informed consent.

Blood Tests

Blood samples were obtained just before treatment and stored at −80°C. HCV-RNA levels before therapy were treated by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems, CA) [Doglio et al., 1999]. Serum HCV-RNA every 2 or 3 month after the initiation of therapy in IFN group was examined by the qualitative PCR assay or RT nested PCR. The lower detection limit of the qualitative assay is 100 copies/ml. HCV genotype was examined by PCR, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously [Dusheiko et al., 1994].

Follow-Up Protocol

Follow-up began on the first day of IFN treatment. In control group, follow-up began on the first day of herbal medicines. Clinical evaluation and biochemical and hematological tests were undertaken at monthly intervals. Twenty-one patients were lost to follow-up. Because HCC did not develop in these 21 patients, they were removed from the subject of this study at the time of final consultation in statistical analysis [Harrington and Fleming, 1983]. Deaths unrelated to HCC and patients who started a new treatment in combination with corticosteroids, immunosuppressive agents, or antiviral drugs during the follow-up were also classified as withdrawals and removed from the study. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings on computed tomography and ultrasonography. Microscopic examination of fine-needle biopsy material was carried out in patients whose angiograms did not demonstrate a typical image of HCC.

Liver Histology

Liver biopsy specimens were obtained percutaneously or by peritoneoscopy 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-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas. Histopathological interpretations of specimens were made independently by experienced liver pathologists (YA and HK) who had no clinical information. Baseline liver histology of chronic hepatitis prior to IFN therapy was classified according to the extent of fibrosis, into three stages: mild, periportal expansion; moderate, portoportal septa; and severe, portocentral linkage, or bridging fibrosis [Desmet et al., 1994]. Patients with severe fibrosis were considered as pre-cirrhosis or cirrhosis.

Statistical Analysis

Baseline characteristics and treatment differences of both groups were analyzed using Fisher's exact test or Krusukall Wallis test. HCC appearance rates were analyzed by the log rank test. A Cox proportional hazards model was used to analyze the factors contributing to the rate of development of HCC: factors examined included age, gender, histologic findings, HCV genotype, HCV load, aspartate aminotransferase (AST), ALT, and AFP. A P-value of <0.05 were considered statistically significant. The SPSS software package (SPSS 11.0 for windows; SPSS, Inc., Chicago, IL) was used for analyses.

RESULTS

Pretreatment Clinical Characteristics

Table I shows the characteristics of the patients with and without IFN treatment. There were no significant differences between the two groups with regard to sex ratio, age, histopathological stage of the liver, serum HCV-RNA level, and AST, ALT, AFP, and blood cell counts. In the control group, eighty-two patients started IFN during follow-up.

Changes in Serum AST, ALT, and AFP Activity After Treatment

Figure 1 shows the serum AST and ALT levels after initiation of treatment. The serum AST and ALT levels declined to normal levels after initiation of treatment with IFN. Transaminase levels at 6, 12, 18, and 24 months after in the IFN group were lower than that of patients not treated with IFN group with statistical significance. Figure 2 shows change in serum AFP level after initiation of treatment. Serum AFP level decreased after the initiation of IFN therapy compared the untreated group with statistical significance.

Loss of HCV-RNA

Of 120 patients treated with IFN, 18 patients lost serum HCV-RNA during IFN treatment. Of the 96 patients who stopped IFN therapy, 8 patients lost HCV-RNA in the serum 6 month after the termination of IFN treatment.

Cumulative Rates of HCC

HCC was diagnosed in four patients in the IFN group and 38 in patients no treated with IFN. Figure 3 shows the cumulative HCC development rates in both groups. The 5- and 10-year cumulative rates of HCC were 5.9 and 13.7%, and 17.1 and 32.8%, for IFN- and no-IFN groups, respectively. The cumulative rate of development of HCC in the IFN group was significantly lower than that not treated with IFN ($P = 0.045$).

Risk Factors for the Development of HCC

The rate of development of HCC after initiation of treatment, Cox regression analysis was performed using several variables. Univariate analysis showed that the following four factors affected significantly the cumulative development of HCC in all patients: histopathological staging ($P < 0.0001$), serum AFP level at 0.5 year after IFN therapy ($P = 0.005$), sex ($P = 0.006$), and IFN therapy ($P = 0.045$) (Table II). The variables were correlated mutually and multivariate Cox regression

TABLE I. Clinical Profiles Before Treatment

Characteristic	IFN group	Non-IFN group	P-value
N[a]	120	240	
Sex (M/F)	65/55	130/110	1
Age (years)[b]	63(60–75)	63(60–75)	1
Liver fibrosis (mild/moderate/severe)[a]	36/40/44	82/70/88	0.965
Genotype (1/2)[a]	85/31	170/61	0.782
HCV-RNA (KIU/ml)[b]	680(10–5,000)	720(5–5,000)	0.176
AST (IU/L)[b]	71(26–446)	67(20–355)	0.493
ALT (IU/L)[b]	86(38–699)	78(46–374)	0.101
AFP (ng/ml)[b]	10(3–316)	9(2–190)	0.342
Hemoglobin (g/dl)[b]	13.7(10.9–17.0)	14.0(11.8–17.0)	0.167
Platelet ($\times 104/mm^3$)[b]	12.8(5.2–25.6)	13.1(5.6–23.2)	0.275
WBC ($\times 103/mm^3$)[b]	4.0(2.1–11.3)	4.0(2.6–7.9)	0.570

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; WBC, white blood count; Normal reference ranges ≤ 10 ng/ml for AFP, 6–50 IU/L for ALT, 11–38 IU/L for AST.
[a]Data are number of patients.
[b]Data are median (range).

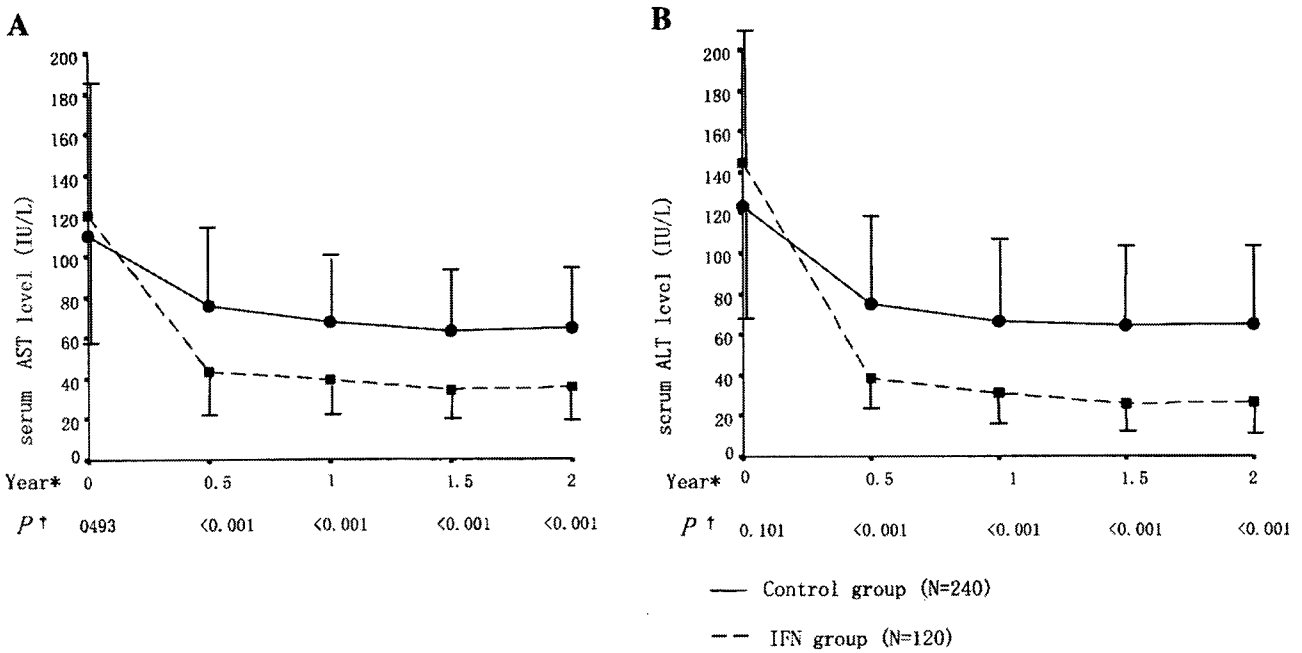

Fig. 1. Changes in serum AST (**panel A**) and ALT (**panel B**) after initiation of treatment. Data were expressed as mean ± standard deviation. *Year after initiation of treatment, †differences of both group by Krusukall Wallis test.

analysis was carried out with the four statistically significant variables in the model (Table III). The development of HCC occurred significant by when: (1) histological staging was advanced, (2) serum AFP level after the initiation of treatment was >10 ng/ml, and (3) IFN was not given. The relative risk of HCC in patients of the IFN group was 0.3 times of that in patients of no-IFN-group (Fig. 3). The relative risk for the development of HCC in patients with severe fibrosis was 3.9 compared to patients with mild or moderate fibrosis. Figure 4 shows the rate of development of HCC based on the difference of treatment and histological staging. IFN therapy could reduce significantly the development of HCC in severe fibrosis (Figure 4, Panel B).

Safety and Tolerance of IFN

Of the 120 patients included in this study, 9 discontinued IFN therapy because of adverse events: 3 cases of general fatigue, 2 cases of psychiatric disorder, 2 cases of aggravation of diabetes mellitus, 1 patient each with thrombocytopenia, pneumonia, and Parkinson's syndrome. The cumulative dropout rate because of IFN-related side effects is plotted in Figure 5. The onset of IFN-related side effects ranged from 204 to 1,569 days after initiation of IFN therapy. These side effects in nine patients disappeared 1 month after cessation of IFN therapy. None of the other patients developed serious side effects that required discontinuation of IFN.

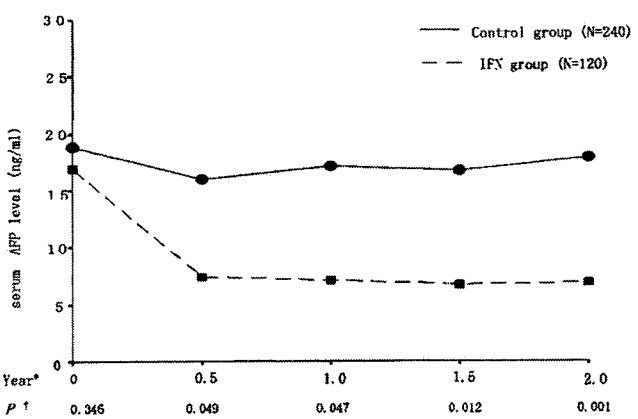

Fig. 2. Changes in serum AFP after initiation of treatment. *Year after initiation of treatment, †differences between IFN group and no-IFN group by the Krusukall Wallis test.

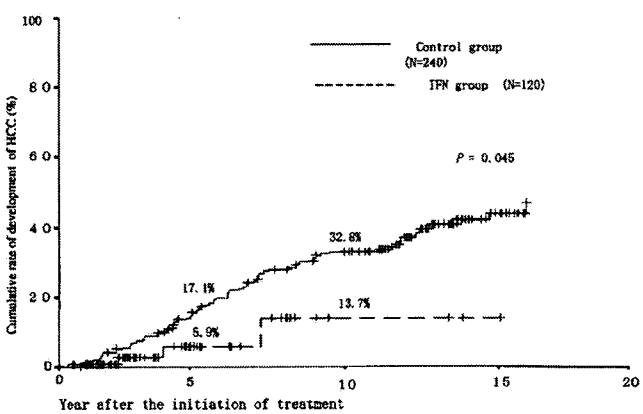

Fig. 3. Cumulative rate of development of hepatocellular carcinoma (HCC) based on the difference in treatment.

TABLE II. Factors Associated With the Development of HCC After Initiation of Treatment by Univariate Cox Regression Analysis

Factor	Category	Odds ratio	95% confidence interval	*P*-value
AFP after treatment (ng/ml)[a]	≤10	1	2.34–7.00	<0.0001
	>10	4.05		
Liver fibrosis	Mild or moderate	1	2.86–7.24	<0.0001
	Severe	4.55		
Sex	Male	1	0.34–0.84	0.006
	Female	0.532		
IFN therapy	−	1	0.13–0.98	0.045
	+	0.35		
AFP (ng/ml)[b]	≤10	1	0.45–36.90	0.209
	>10	2.23		
ALT after treatment[a]	≤50	1	0.53–6.29	0.342
	>50	1.82		
ALT (IU/L)[b]	≤100	1	0.73–2.34	0.371
	>100	1.31		
Age (years)[b]	≤65	1	0.23–11.78	0.615
	>65	1.65		
HCV-RNA after treatment[a]	−	1	0.29–14.88	0.474
	+	2.06		
BMI[b]	≤25	1	0.08–7.29	0.800
	>25	0.74		
HCV-RNA (KIU/ml)[b]	>100	1	0.05–4.21	0.490
	≤100	0.57		
HCV genotype[b]	1	1	0.45–2.49	0.907
	2	1.05		

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BMI, body mass index; HCC, hepatocellular carcinoma; IFN, interferon.
[a]Serum level 6 months after initiation of treatment.
[b]Measurement at initiation of treatment.

DISCUSSION

Several findings from the present study have direct implications for long-term IFN treatment in aged patients with chronic hepatitis or cirrhosis. First, the AFP baseline was decreased after initiation of IFN therapy in most patients. Second, the cumulative HCC development rate in patients whose serum level of AFP was within normal limits after initiation of IFN therapy was lower than that of patients with high level of AFP despite of IFN therapy. These suggest that AFP is a suitable indicator in long-term IFN therapy for protecting against HCC. If long-term IFN therapy could maintain normalization of serum AFP level, HCC development could be prevented in HCV patients.

AFP is a glycoprotein produced by the liver or yolk sac in fetal life in vertebrates, and it is not normally present in the serum of adults and is used commonly as a tumor marker for HCC [Otsuru et al., 1988]. Many reports have cited elevated AFP baselines as an independent HCC risk factor together with age, gender, liver histology stage, and ethnicity in patients infected with HCV [Ikeda et al., 1993; Tsukuma et al., 1993]. Elevation of AFP has been observed after a rise in transaminase in acute hepatitis, fulminant hepatitis, and acute exacerbation of chronic hepatitis. This type of AFP elevation is explained as a result of hepatocyte regeneration accompanied by necroinflammatory changes [Hu et al., 2004]. On the other hand, Yoshida et al. [2002] have reported that the HCV-coding core

TABLE III. Factors Associated With the Development of HCC After Initiation of Treatment by Multivariate Cox Regression Analysis

Factor	Category	Odds ratio	95% confidence interval	*P*-value
Liver fibrosis	Mild or moderate	1	1.69–6.13	<0.0001
	Severe	3.89		
AFP after treatment (ng/ml)[a]	≤10	1	1.45–4.84	0.002
	>10	2.65		
IFN therapy	−	1	0.11–0.86	0.025
	+	0.304		

AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; IFN, interferon.
[a]Serum AFP level 6 months after initiation of treatment.

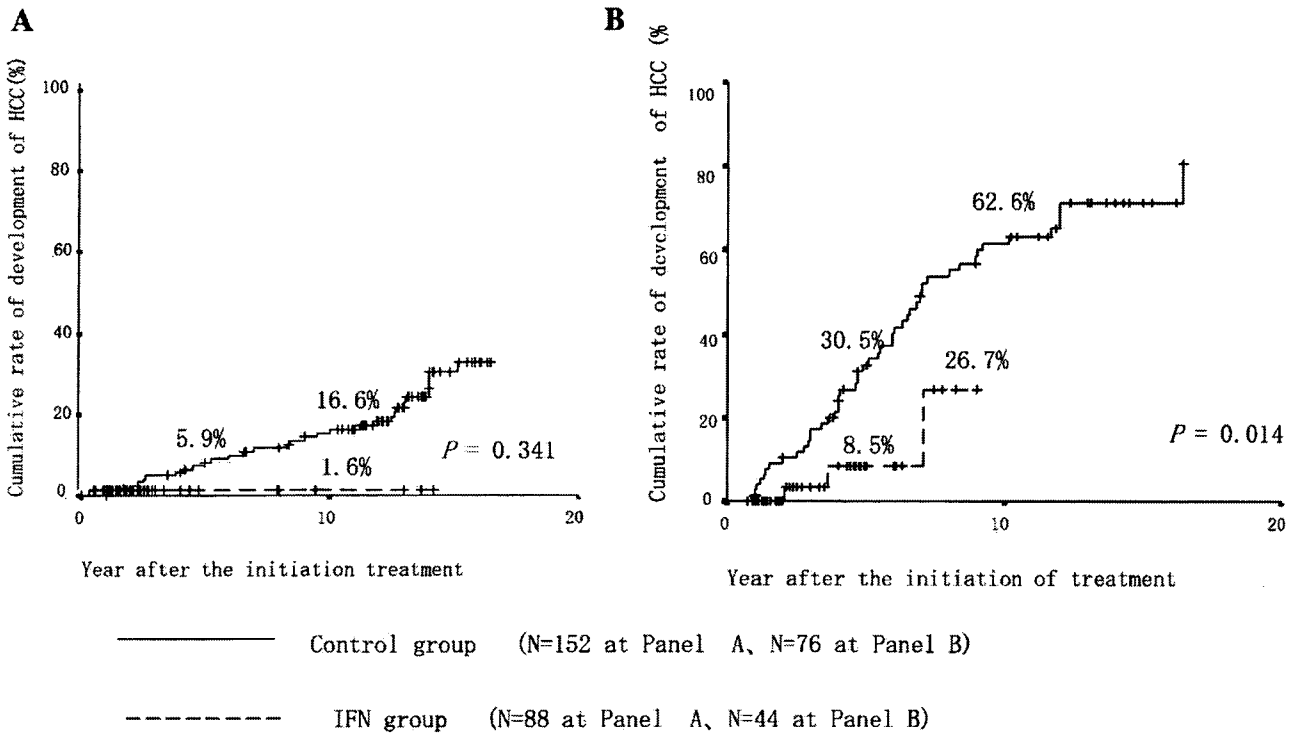

Fig. 4. Cumulative rate of development of hepatocellular carcinoma (HCC) based on the difference of treatment and histological staging (**Panel A**); patients with mild or moderate fibrosis. **Panel B**: patients with severe fibrosis.

protein is related to the cell cycle and cell proliferation at the transcriptional level in hepatocytes. This might mean that the HCV-coding core protein upregulate AFP production in hepatocytes.

AFP has the following functions associated with the hepatocarcinogenesis. First, AFP plays a role in the carrier-transport of various ligands and binds to a large variety of ligands such as fatty acids and estrogens. The action of AFP ligands can lead to cells toward multiplication or differentiation [Parmelee et al., 1978; Jacobson et al., 1990; Deutsch, 1991]. Second, AFP could control cell growth. AFP synergies growth factors such as epidermal growth factor and insulin like growth factor to cause proliferation of granulose cells. AFP has been found to regulate the proliferation of human mammary tumor cells [Wang and Alpert, 1995]. Wang and Xu [1998] have reported that human AFP can enhance the mouse hepatoma H-22 and human hepatoma SNMC-7721 cells in vitro. Third, AFP has been found to have immunosuppressive activity. AFP also suppresses the natural killer cell activity and induces suppressor T cells. AFP prevents the expression of MHC-II class molecule on macrophages [Lester et al., 1976].

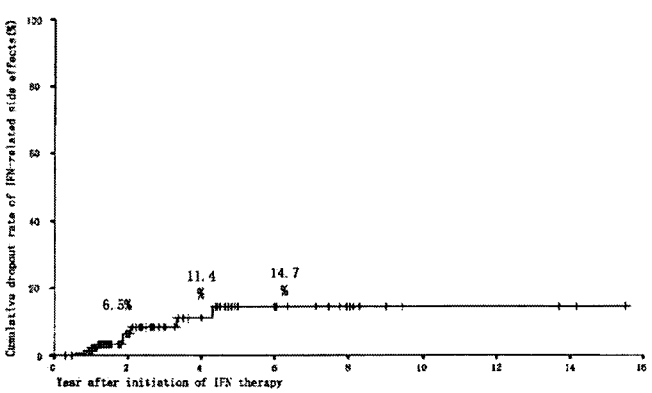

Fig. 5. Cumulative dropout rates due to IFN-related side effect.

Subsequently, Wang et al. [2001] has reported that antisense phosphorothioate oligodeoxyribonucleotide targeted to AFP genes inhibit the growth of human hepatoma cells and solid hepatoma, which is related to their cell apoptosis induction. As described above, elevated AFP might be associated with the hepatocarcinogenesis. Murashima et al. [2006] have reported that the competing action of IFN against HCV-related protein may cause decrease the production of AFP. Thus, normalization of serum AFP level by prolonged-IFN therapy could also protect against the development of HCC.

IFN therapy could reduce significantly the development of HCC in severe fibrosis. Long-term IFN treatment can be associated with serious side effects and is costly. Accordingly, careful selection of patients for long-term IFN therapy is important. The development rate of HCC is high in aged patients with severe liver fibrosis and elevated AFP. On the other hand, the development of HCC is low in no-aged patients with non-severe fibrosis and low level of AFP. Therefore, long-term IFN treatment for protection against HCC could be

recommended for patients with elevated AFP level and/or severe liver fibrosis who can tolerate IFN-related side effects. Considering cost-effectiveness, it seems reasonable to select aged patients with elevated AFP and/or severe fibrosis for long-term IFN therapy for protecting against the development of HCC.

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Interferon and lamivudine monotherapy on chronic hepatitis B in Japan

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Aim: We show data of interferon (IFN) and lamivudine monotherapy on chronic hepatitis B in Japan.

Methods: Data collected from sixty-six chronic hepatitis B (CHB) Japanese patients who were treated with IFN for 6 months were analyzed. The efficacy of long-term IFN therapy in 52 patients with e-antigen positive CHB, and data from 290 chronically HBV-infected patients who were treated with lamivudine for more than 3 years, were analyzed.

Results: Six-month IFN therapy: among 45 patients with HBeAg at commencement of IFN therapy, nine (20%) were responders. Young patients especially those with high serum alanine aminotransferase (ALT) levels were much more likely to respond to IFN therapy. Twelve-month IFN therapy: the response rate was 31% among 52 patients with HBeAg. Long-term lamivudine therapy: YMDD motif mutation was detected in 167 of 290 patients (58%) during lamivudine treatment. Breakthrough hepatitis from lamivudine resistant virus was detected in 93 of 290 patients (32%). Finally, 813 patients were treated by lamivudine between September 1995 and February 2006. Fifteen patients lost HBsAg during and after lamivudine therapy.

Conclusion: Long-term interferon therapy has a better response than short-term interferon therapy. Some patients lost HBsAg during and after lamivudine therapy.

Key words: HBV, interferon, lamivudine

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a common disease that can lead to a chronic carrier state, and is associated with risk of development of progressive disease and hepatocellular carcinoma.[1] Interferon (IFN) and lamivudine are two currently approved treatments for chronic hepatitis B (CHB) in most countries.[2] IFN is associated with significant adverse effects, and long-term therapy with lamivudine may result in drug resistance. A meta-analysis of IFN therapy published in 1993 reviewed 15 randomized controlled studies involving 837 adult patients who received IFN-α in doses of 5–10 million units (MU) administered daily to three times weekly for 4–6 months.[3] Loss of hepatitis B e-antigen (HBeAg) occurred in 33% of treated patients compared with 12% of controls. Loss of detectable HBV DNA and normalization of alanine aminotransferase (ALT) levels were also more common in treated than control patients. The main pretreatment factors that correlated with a response were high ALT levels,[4–6] low HBV DNA,[4,5] female sex, and greater degrees of activity and fibrosis on liver biopsy.[2] However, the optimal duration of IFN therapy for CHB is not well established. Moreover, the duration of IFN therapy was mainly one month in the 1990s in Japan and the efficacy was limited.[7–9]

Several studies have reported the effectiveness of some nucleoside analogs such as lamivudine[10–12] in the suppression of HBV replication, improvement of transaminase levels and liver histology, and enhancement of the rate of loss of HBeAg.[13] However, in patients who do not show loss of HBeAg, cessation of therapy after 3–12 months could potentially be associated with return to pretreatment HBV DNA levels and relapse of the disease.[14,15] Considering the safety of lamivudine, it has been suggested that continuous therapy may be beneficial, particularly in patients who do not show HBeAg seroconversion.[16] Leung *et al.*[17] showed that after 3 years of continuous treatment with lamivudine, 40% of patients achieved HBeAg seroconversion.

A large problem with long-term use of lamivudine, however, is the potential development of viral resistance, associated with increases in HBV DNA and serum transaminases. Resistance to lamivudine often develops after 6 months of treatment[18,19] and is associated with mutations in the HBV polymerase gene. Resistance was recently reported to develop in 15 and 38% of patients

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after 1 and 2 years of treatment, respectively.[20] Therefore, long-term lamivudine therapy may increase the likelihood of development of resistance.

Recently, HBV genotypes have been implicated in HBeAg seroconversion as well as response to antiviral treatment. Genotype A was found to be associated with a higher rate of IFN-induced HBeAg seroconversion than genotype D in a study of 64 German patients with HBeAg-positive CHB.[21] Another study of 58 Taiwanese patients who received IFN treatment for HBeAg-positive CHB found that genotype B had a significantly higher rate of HBeAg loss compared with those of genotype C.[22] Our previous study indicated that in Japan, the proportions of HBV infection associated with genotypes B and C are 9 and 88%, respectively.[23] Our study also showed that most genotype B cases were HBeAg-negative at first examination and showed a mild degree of hepatic fibrosis, while genotype C infection was associated with progressive liver fibrosis.[23] Therefore, mainly patients with genotype C of CHB have received antiviral treatment in Japan.

We show data for IFN and lamivudine monotherapy on CHB in Japan. Some present studies have been published.[24,25]

INTERFERON THERAPY

Six-month IFN therapy

WE ANALYZED 66 CHB Japanese patients who were treated with IFN for 6 months. They comprised patients who were HBeAg positive ($n = 45$) and negative ($n = 21$). One (2%), 8 (13%), and 51 (85%) patients were infected with hepatitis B virus genotypes A, B and C, respectively. Responders were defined as patients positive for HBeAg who showed normalization of serum ALT level, HBeAg loss and HBV DNA negativity 6 months after completion of IFN therapy. In patients negative for HBeAg, responders were defined as patients who showed normalization of ALT level and HBV DNA negativity at the same point.

Among 45 patients with HBeAg at commencement of IFN therapy, 9 (20%) were responders. Young patients, especially those with high serum ALT levels, were more likely to respond to IFN therapy. Among 21 patients negative for HBeAg, 13 (62%) were responders. There were no significant differences ($P = 0.0048$ and $P = 0.049$, respectively) in clinical characteristics between responders and non-responders among patients negative for HBeAg. Multivariate analysis identified HBeAg negativity and young age as independent factors associated with positive response to 6-month IFN therapy. However, long-term follow-up of treated patients showed a fall in the response rate.[24] We analyzed the rate of HBsAg clearance caused by IFN therapy. The cumulative percent of patients who were cleared of HBsAg was analyzed. The clearance rate of HBsAg at 5 years was 4% and at 10 years was 11%.

Twelve-month IFN therapy

We evaluated the efficacy of long-term IFN therapy in patients with e-antigen positive CHB. This study design was a prospective, randomized controlled clinical trial.[25] Fifty-three patients were randomly assigned into one of two groups, treated with 3 MU of IFN (low dose group, $n = 27$) or 6 MU IFN (high dose group, $n = 26$), administered twice weekly for 52 weeks. Responders were defined as patients positive for HBeAg who showed normalization of serum ALT level, HBeAg loss and HBV DNA negativity 6 months after completion of IFN therapy. One patient in the high dose group dropped out because of transfer. The remaining 52 patients were examined by intention-to-treat (ITT) analysis. The response rates by ITT analysis were 40.7% (11/27) in the low dose and 20% (5/25) in the high dose groups. The difference between low and high dose groups was not statistically significant. Univariate analysis of clinical factors that contribute to the response demonstrated that IFN therapy had a significant effect when the serum HBV DNA level was <200 Meq/mL prior to the commencement of IFN therapy ($P = 0.033$). Transient acute exacerbation of ALT was present during or after IFN therapy ($P = 0.031$). Multivariate analysis showed that the risk ratio for the development of response in patients with serum HBV DNA levels less than 200 Meq/mL was 3.60 compared with patients with ≥200 Meq/mL.

LAMIVUDINE THERAPY

WE STUDIED 813 Japanese adult patients (164 females and 649 males) who commenced treatment with lamivudine between September 1995 and February 2006 and adhered to the treatment at the Department of Hepatology of Toranomon Hospital. In these 813 patients, 290 who received lamivudine treatment over 3 years (median 55 months) were investigated. Among the 290 patients, 239 were male with a median age of 44, chronic hepatitis was present in 248 patients, and 132 were HBeAg positive. Eight (3%), 24 (8%), 249 (86%) patients were infected with hepatitis B virus genotypes A, B and C, respectively. All patients

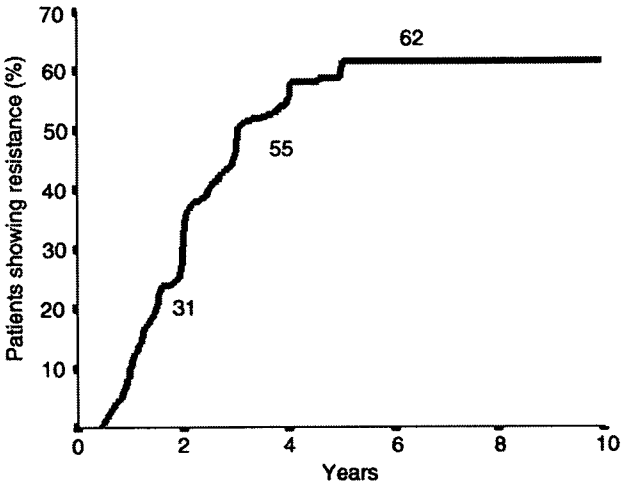

Figure 1 Cumulative percent of patients who exhibited viral resistance during treatment with lamivudine (Kaplan–Meier method).

were followed up from commencement of therapy at our hospital and had been treated continuously. If treatment was discontinued and re-commenced at a later date, we analyzed only the first round of therapy. Some patients have been reported previously.[13,19,26–37] All patients had detectable HBsAg and HBV DNA for more than 3 months prior to commencement of lamivudine therapy. All patients had elevated serum ALT for 3 months before commencement of therapy. No patients had hepatocellular carcinoma at the commencement of therapy. Chronic hepatitis or cirrhosis was confirmed by needle biopsy, peritoneoscopy or clinical criteria before treatment.[29] Two hundred and forty-eight and 42 patients were diagnosed with chronic hepatitis and cirrhosis, respectively.

In this study YMDD motif mutation was detected in 167 of the 290 patients (58%) during the treatment of lamivudine. Figure 1 shows the cumulative percent of patients who exhibited emergence of mutations during treatment with lamivudine. The frequency of emergence of mutations gradually increased. Moreover, patients with HBeAg at the commencement of treatment had a higher rate of emergence of mutation by the Kaplan–Meier method ($P = 0.013$). In this study, breakthrough hepatitis caused by lamivudine resistance was detected in 32% of patients (93/290). Figure 2 shows the cumulative percent of patients who developed breakthrough hepatitis. The frequency of breakthrough hepatitis gradually increased. Patients with HBeAg at the commencement of the treatment had a higher rate of breakthrough hepatitis by the Kaplan–Meier method ($P = 0.0066$). We analyzed the cumulative percentage of genotype A, B and C patients who experienced mutations and developed breakthrough hepatitis. Rates of both the emergence of lamivudine resistance and the occurrence of breakthrough hepatitis were the highest in genotype A patients, next genotype C, and lowest in genotype B.

Among 93 patients who had breakthrough hepatitis, 63 received antivirus drugs (adefovir dipivoxil, entecavir and IFN). The efficacy of lamivudine therapy involving these other antiviral drugs was investigated. At the commencement of treatment, 132 patients were HBeAg positive. The proportion of these patients who achieved HBeAg loss was 40% at 1 year, 53% at 3 years and 73% in 5 years. Alternatively, at the commencement of treatment, 158 patients were HBeAg negative. The rates of ALT normalization were approximately 90% at all points from 1 to 5 years. Undetectable rates of HBV DNA during lamivudine therapy were approximately 70–80% at all points.

Finally, 813 patients were treated by lamivudine between September 1995 and February 2006 in Toranomon Hospital. Among these 813 patients, 15 lost HBsAg during and after lamivudine therapy.

DISCUSSION

ALTHOUGH IFN IS reported to have beneficial effects in chronic hepatitis B, the response rate is not high. Kao *et al.*[22] reported that HBV genotype C,

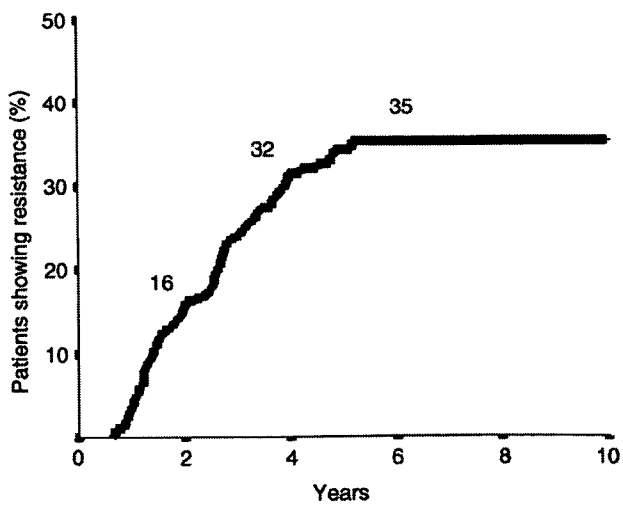

Figure 2 Cumulative percent of patients who developed breakthrough hepatitis during treatment with lamivudine (Kaplan–Meier method).

compared to genotype B, is associated with a lower response rate to IFN-α therapy among CHB with HBeAg. The response rate among our patients with genotype C was low, similar to the results of Kao *et al.*[22] (15% response rate). In our study, young patients especially those with high ALT levels at baseline were more likely to respond to IFN among HBeAg positive patients. These factors were similar to those reported in previous studies.[4–6] We showed that 31% (16/52) patients who received IFN-α given twice per week for 52 weeks were responders.[25] Therefore, a long-term therapeutic regimen may be necessary to secure a better response than than short-term therapy.

The response rate in patients negative for HBeAg was higher than in those with HBeAg. Previous reports showed that the response rate to a 6–12-month course of IFN-α in patients with HBeAg-negative CHB ranged 10–47% (24% average).[38–41] Moreover, our previous report showed that 75% (9/12) patients who received IFN-β given twice per week for 24 weeks responded to therapy.[42] Considered together, the efficacy of IFN in patients negative for HBeAg is high. However, the factors that could predict a sustained response are less well defined in HBeAg negative than positive patients.[2] The dose of IFN also had little effect, but duration of therapy (12 *vs* 5–6 months) was associated with doubling of sustained response rates.[43]

We analyzed the efficacy of lamivudine treatment over 3 years. Our previous study[29] demonstrated the effects of lamivudine therapy in Japanese patients with HBV infection. Patients with genotype B and/or HBeAg negative HBV infection had better responses to lamivudine therapy compared to patients with genotype C and HBeAg positive disease. This study also showed the same result. Although rates of the emergence of lamivudine resistance and the occurrence of breakthrough hepatitis gradually increased, the efficacy of lamivudine therapy involving these other antiviral drugs was better. Moreover, some patients lost HBsAg during and after lamivudine therapy. Thus, some patients showed good response and can discontinue lamivudine therapy.

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Original Article

Evaluation of long-term biochemical responses to combination therapy of interferon plus ribavirin in those infected with hepatitis C virus genotype 1b and high baseline viral load

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Aim: The aim of this study was to determine the long-term effects in non-responders (NRs) to 48-week interferon (IFN) and ribavirin combination treatment in patients infected with hepatitis C virus (HCV) genotype 1b and high baseline viral loads.

Methods: We measured serum alanine aminotransferase (ALT) and HCV-RNA levels in 52 consecutive patients infected with HCV genotype 1b and high viral loads who received combination therapy for 48 weeks.

Results: Sustained virologic response (SVR) was noted in 30 patients (57.7%). Virologic response (VR), that is serum HCV-RNA negativity by the end of treatment and positivity during follow-up, was noted in nine patients (17.3%). Thirteen (25.0%) patients were NRs. Significantly lower serum albumin ($P = 0.007$) and ribavirin doses according to body weight ($P = 0.021$) and higher gamma glutamyl transpeptidase (GGT, $P = 0.038$) were noted at baseline in the NR group than in the SVR and VR groups. ALT normalization rates at six months after the completion of treatment were 55.6% (5/9) in VR and 61.5% (8/13) in NRs. Sustained ALT normalization at two years after the completion of treatment was noted in 55.6% (5/9) and 58.3% (7/12), respectively.

Conclusion: Our study indicates a high rate of ALT normalization in patients infected with HCV genotype 1b and high baseline viral loads who received combination therapy and that such a rate could be maintained after the completion of therapy, even in NRs. Our results suggest that combination therapy should be continued in NRs who show ALT normalization in order to prevent potential hepatocarcinogenesis.

Key words: ALT, chronic hepatitis C, combination therapy, non-responder, ribavirin

INTRODUCTION

HEPATITIS C VIRUS (HCV) usually causes chronic infection and is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC).[1–3] The aim of any treatment for chronic hepatitis C is to delay the progression of liver fibrosis and inhibit the development of HCC by the eradication of HCV, and normalization of alanine aminotransferase (ALT), even if viral clearance cannot be achieved.[4]

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The combination therapy of interferon (IFN) α-2b or pegylated-IFN (PEG IFN) α-2b plus ribavirin is the first-line therapy for patients with chronic hepatitis C. The addition of ribavirin to IFN or PEG IFN is reported to enhance the virological response even in "IFN-resistant" patients.[5–7] The achieved sustained virological response (SVR) rate by 48-week combination therapy was approximately 50% and significantly higher than in patients who received 24- or 48-week IFN monotherapy.[8,9] However, some patients, particularly those with genotype 1b and high viral load, fail to respond to treatment and cannot achieve viral clearance even following combination therapy.

Recently, Iino *et al.*[10] reported that sustained ALT normalization (ALT normal at 24 weeks after the end of treatment) following combination therapy was 28.1%,

which was higher than the 10.5% with IFN monotherapy. Their results suggested that combination therapy might inhibit the progression to HCC, based on the high SVR rate and high rate of sustained ALT normalization.[10]

The aims of the present study were to determine the non-virological response (no-response, NR) rate and evaluate ALT normalization during therapy and at long-term follow-up in patients with high viral load and chronic infection with HCV genotype 1b who received combination therapy of IFN α-2b or PEG IFN α-2b plus ribavirin.

METHODS

Patients

FROM DECEMBER 2001 to February 2006, a total of 425 Japanese patients with chronic HCV infection caused by genotype 1b and high baseline viral loads received combination therapy of IFN-α-2b or PEG IFN α-2b plus ribavirin for 48 weeks at Toranomon Hospital, Tokyo, Japan. Among them, 52 patients who could be followed up for more than one year after the completion of 48 week-combination therapy were selected for the present retrospective study. The inclusion criteria were: (i) a positive test for anti-HCV antibody; (ii) HCV genotype 1b (confirmed by a PCR-based method[11]); (iii) serum HCV-RNA levels more than 100 KIU/mL by quantitative PCR assay (Amplicor GT-HCV Monitor version 2.0; Roche Diagnostic Systems, Pleasanton, CA) within the preceding 12 weeks (defined as "high" viral load); (iv) persistently high serum ALT concentrations (the upper limit of normal for ALT is 50 IU/L) during the preceding 12 weeks; (v) a diagnosis of chronic hepatitis on liver biopsy specimen obtained within the preceding one year of enrollment; (vi) hemoglobin concentration of ≥12.0 g/dL; (vii) platelet count of $\geq 100 \times 10^3/\mu l$; and (viii) signing a consent form of the study protocol that had been approved by Human Ethics Review Committee of Tranomon Hospital. Patients with the following conditions were excluded from the study: (i) other forms of liver disease (e.g. primary biliary cirrhosis, alcoholic liver disease and autoimmune liver disease); (ii) treatment with any other antiviral or immunomodulatory agents administered within the preceding 24 weeks; (iii) patients with hepatitis B surface antigen or hepatitis B core antibody; (iv) coinfection with human immunodeficiency virus; and (v) women who were pregnant or lactating. The selected subjects included 33 males and 19 females, aged 19–65 years, with a median age of 52 years.

Study protocol

The combination treatment was provided for 48 weeks, with a subsequent follow-up period of more than one year. In 21 (40.3%) patients, IFN α-2b (Schering-Plough, Osaka, Japan) was injected intramuscularly at 6 million units (MU)/day for the initial two weeks, followed by three times per week for 46 weeks. In the remaining 31 (59.6%) patients, 1.5 μg/kg/week of PEG IFN α-2b (Schering-Plough, Osaka, Japan) was injected subcutaneously for 48 weeks. All patients received ribavirin (Schering-Plough, Osaka, Japan) at a dose adjusted to body weight (600 mg for individuals with body weight of ≤60 kg, 800 mg for weight of 60–80 kg, and 1000 mg for weight ≥80 kg).

Biochemical and virological responses to treatment were assessed during the 48-week treatment period and during the subsequent follow-up period. Biochemical response was defined as normalization of serum ALT activity (the upper limit of normal for ALT is 50 IU/L) by the end of treatment. Virological response (VR) was defined as undetectable serum HCV-RNA at the end of the 48-week treatment, as confirmed by a qualitative PCR assay (Amplicor HCV version 2.0; Roche Molecular Systems, Belleville, NJ), but reappearance of HCV-RNA during the one-year follow-up period. A sustained virological response (SVR) was defined as disappearance of serum HCV-RNA after the completion of treatment until the end of the follow-up period. A non-virological response (NR) was defined as persistent presence of HCV-RNA during treatment.

Blood tests

Routine biochemical and hematological tests were performed at least once every month during and after treatment. Serum HCV-RNA levels were measured using a quantitative PCR assay with a lower detection limit of quantification of 0.5 KIU/mL (Amplicor HCV Monitor version 2.0; Roche Diagnostics, Pleasanton, CA). The presence or absence of serum HCV-RNA was assessed using a qualitative PCR assay (Amplicor HCV version 2.0; Roche Diagnostics, Pleasanton, CA) with a lower detection limit of 100 copies/mL.

Liver histopathological examination

Histopathological staging of liver biopsy specimens obtained at baseline and during treatment was performed according to the classification of Desmet *et al.*[12]

Table 1 Comparison of baseline clinical profiles of patients infected by HCV genotype 1b and a high viral load with or without viral disappearance during combination therapy

	Total ($n = 52$)	SVR + VR ($n = 39$)	NR ($n = 13$)	*P*-value
Age (y)†	19–65 (52)	19–64 (51)	37–65 (53)	0.808
Sex (M/F)	33/19	26/13	7/6	0.501
Body weight (kg)	42.3–120 (62.4)	46–77 (63.1)	42.3–120 (60)	0.759
Ribavirin dose/kg weight (mg/kg)	6.7–14.2 (11.2)	10.1–13.1 (11.4)	6.7–14.2 (10.7)	0.021
Histopathological staging (F1/2/3)	28/18/6	22/14/3	6/4/3	0.322
Histopathological grading (A1/2/3)	28/24/0	22/17/0	6/7/0	0.541
ALT (IU/L)	29–276 (98)	29–276 (101)	50–135 (71)	0.131
GGT (IU/L)	16–240 (62)	16–240 (60)	40–121 (75)	0.038
Hemoglobin (g/dL)	12.0–17.4 (14.4)	12.0–17.4 (14.5)	12.5–16.3 (14.4)	0.674
Platelet count ($\times 10^3/\mu L$)	101–309 (177)	101–309 (178)	111–237 (173	0.550
Fe (μg/dL)	46–308 (139)	46–308 (143)	70–214 (130)	0.393
Ferritin (μg/L)	<10–644 (176)	<10–644 (188)	52–335 (125)	0.290
ICG R15 (%)	7–41 (15)	7–33 (13)	7–41 (17)	0.842
Albumin (g/dL)	3.4–4.5 (3.8)	3.4–4.5 (3.9)	3.4–3.9 (3.7)	0.007
HCV-RNA (KIU/mL)	49–3500 (795)	110–3500 (810)	49–2800 (940)	0.512
Follow-up period (month)	18–43 (38)	18–43 (38)	18–42 (39)	0.617

†Data are ranges (median).
ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; ICG R15, indocyanine green retention rate at 15 min; NR, non-responders; SVR, sustained virological response; VR, virological response.

Statistical analysis

Nonparametric tests, including the χ^2, Fisher's exact probability and Mann–Whitney *U*-tests, were used to analyze the baseline clinical profile of patients. Univariate and multivariate logistic regression analyses were used to determine the factors that significantly contributed to a VR. We also calculated the odds ratios and 95% confidence intervals (95% CI). A *P*-value < 0.05 by the two-tailed test was considered statistically significant. All analyses were performed using SPSS version 10.1 (SPSS, Chicago, IL).

RESULTS

Baseline characteristics

THE BASELINE CLINICAL profile of the patients at commencement of the combination therapy is shown in Table 1. The follow-up period ranged from 18 to 43 months (median, 38 months). The baseline viral load ranged from 49 to more than 5000 KIU/mL (median, 795 KIU/mL).

Virological response rates

Of 52 patients, 39 (75.0%) achieved viral disappearance during the combination therapy, 30 (57.7%) patients attained an SVR and nine (17.3%) patients had a reappearance of HCV-RNA during the follow-up period, that is VR. The remaining 13 (25.0%) were NRs (Table 2). We compared the baseline clinical profile of SVR plus VR group and NR group (Table 1). There were no differences between the two groups with respect to age, sex, body weight, histopathological findings, serum ALT, hemoglobin, platelets count and HCV-RNA levels at the commencement of the combination therapy. Serum albumin concentrations ($P = 0.007$) and ribavirin dose according to body weight ($P = 0.021$) were significantly lower and γ-glutamyl transpeptidase (GGT, $P = 0.038$) was higher in the NR group than in the other group. We then analyzed the data to determine those factors that could predict a VR. Univariate analysis identified three parameters that significantly influenced the VR: serum albumin concentrations ≥3.9 g/dL ($P = 0.012$), GGT < 50 IU/L ($P = 0.048$) and ribavirin dose according to body weight ≥11.2 ($P = 0.009$). Multivariate analysis using variables including sex, age, serum albumin, ALT, GGT and ribavirin dose according to body weight, identified three parameters that independently influenced the virological response: male ($P = 0.035$), ribavirin dose according to body weight ≥11.2 ($P = 0.012$) and GGT < 50 IU/L ($P = 0.010$).

Table 2 ALT normalization in patients infected with genotype 1b and a high baseline viral load, who received 48-week combination therapy of IFN plus ribavirin

	ALT normalization after completion of treatment			
	End of treatment	6 months	1 year	2 years
VR ($n = 9$)	7	5	5	5/9
Follow up period; 17–42 (40) months	(77.8%)	(55.6%)	(55.6%)	(55.6%)
NR ($n = 13$)	10	8	6	7/12†
Follow up period; 19–42 (36) months	(76.9%)	(61.5%)	(46.2%)	(58.3%)
Total		13/21 (59.1%)		12/21† (57.1%)

†One patient could not be followed up for 2 years after completion of treatment.
ALT, alanine aminotransferase; NR, non-responders; VR, virological response.

ALT normalization rate

ALT normalization rate during the 48-week combination therapy is shown in Table 2. We determined the ALT normalization rate for non-SVR, VR and NR, because all SVR cases exhibited ALT normalization after the completion of treatment. ALT normalization rates in patients who showed VR and NR were 77.8% (7/9) and 76.9% (10/13) at the end of treatment, respectively; and 55.6% (5/9) and 61.5% (8/13) at six months after the completion of treatment, respectively. For the same two groups, the rates of sustained ALT normalization at two-years after the completion of treatment were 55.6% (5/9) and 58.3% (7/12), respectively. The overall ALT normalization rate at two years after the completion of treatment is shown in Table 3. Because of the high rate of sustained ALT normalization among the SVR cases ($n = 30$), the overall ALT normalization rate at two years after completion of treatment for all patients was 82.4% (42/51).

Table 3 Rates of SVR, VR and NR, and overall ALT normalization in patients infected with genotype 1b and a high baseline viral load who received 48-week combination therapy of IFN plus ribavirin

	N	Rate		ALT normalization at 2 years after completion of treatment	
SVR	30	57.7%		30/30	100%
VR	9	17.3%	⇨	5/9	55.6%
NR	13	25.0%		7/12†	57.1%
Total	52			42/51†	82.4%

†One patient could not be followed up for 2 years after completion of treatment.
ALT, alanine aminotransferase; NR, non-responders; SVR, sustained virological response; VR, virological response.

Figure 1 shows the clinical course of a patient who showed the persistent presence of HCV-RNA during treatment but achieved sustained ALT normalization after the completion of combination therapy. The HCV-RNA level decreased only 1 log relative to baseline viral load at the end of the combination therapy. However, serum ALT normalized rapidly after the commencement of treatment and was persistently normal over more than three years after the completion of the treatment.

DISCUSSION

THE PRIMARY PURPOSE of treatment of patients with chronic hepatitis C is the eradication of HCV, that is SVR. However, patients who are infected with HCV genotype 1b and have a high baseline viral load are considered refractory to IFN, and the SVR rate in such patients is less than 10%.[9] However, the use of ribavirin in combination with IFN has markedly improved the SVR rate.[5–7,9,13] Early disappearance of HCV-RNA in the serum samples is a predictor of SVR to IFN-based therapy, and the most appropriate time-point for determining the outcome of combination treatment is week

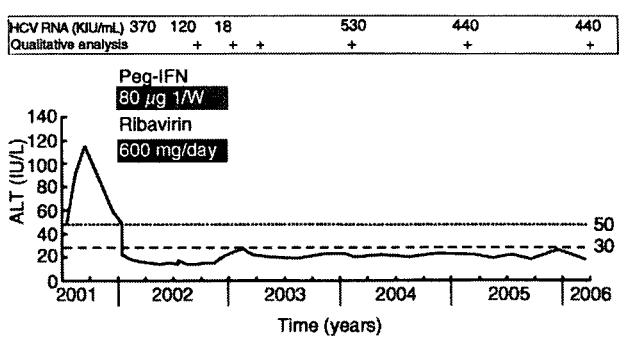

Figure 1 Clinical course of a patient with sustained ALT normalization who showed the persistent presence of HCV-RNA.

12 of such therapy.[14,15] We reported previously that when the loss of HCV occurs at more than 24 weeks, the negative predictive value for SVR was 100%.[14] Consequently, it is not clear how to treat such patients (i.e. those infected with genotype 1b and had a high baseline viral load who are HCV-positive at 24 weeks after the initiation of combination therapy of IFN plus ribavirin).

In the present study, we investigated cases who were HCV-RNA positive during the combination therapy, that is NRs. Compared with patients who were HCV-RNA negative during the combination therapy, our patients had significantly lower serum albumin and higher GGT (Table 1). In multivariate analysis to determine those factors that could predict a virological response, although serum albumin did not independently influence the VR ($P = 0.062$), we consider this result approximately similar to our previous reports, in which we identified serum albumin as a predictor of non-VR in patients on 48 weeks of the same combination therapy.[16,17] The results that GGT independently influenced the virological response ($P = 0.010$) confirm the findings of previous studies that GGT levels correlate with sustained virological response.[18,19] The low level of serum albumin reflects the deterioration of the ability of the liver to synthesize serum proteins due to progression of liver fibrosis. This finding suggests that eradication of HCV-RNA is difficult in patients with advanced liver fibrosis, even when treated with IFN monotherapy or combination therapy.

However, our results showed that even when patients with genotype 1b and high viral load do not achieve HCV-RNA negativity during combination therapy with IFN and ribavirin, they achieved a high rate of ALT normalization (76.9%) at the end of therapy and could maintain a normal ALT level over a long period of time after the completion of combination therapy (58.3% at two years after the completion of such therapy) (Table 2). In IFN monotherapy, the ALT normalization rate reported in genotype 1 patients ranged from 10 to 32%.[4,20,21] We also reported that among 1654 patients treated with IFN alone, 266 (16.1%) showed normal levels of ALT without loss of HCV-RNA for ≥6 months after the completion of IFN monotherapy. Considered together, these results suggest that a higher ALT normalization rate can be achieved by combination therapy.

On the other hand, the SVR rate improved to approximately 57.7% in our 48-week combination therapy, and ALT normalization was noted in approximately 60% of non-SVR cases at six months and 57.1% at two years after completion of combination therapy (Table 2). In other words, the overall ALT normalization rate was extremely high (82.4%). These results are similar to those of a previous study,[10] which showed a higher rate of sustained ALT normalization (normal ALT levels at 24 weeks after the end of treatment) with combination therapy than with IFN monotherapy.

The natural history of chronic hepatitis C includes cirrhosis and hepatocellular carcinoma. Previous studies reported that the predictive factors of progression to cirrhosis from chronic hepatitis C were male sex, heavy alcohol consumption, elevated serum ALT levels and histology of high grade necroinflammatory activity.[22] In this regard, we reported previously that normalization of ALT levels after IFN therapy without loss of serum HCV-RNA was associated with decreased incidence of hepatocarcinogenesis.[23,24] In the present study, we could not investigate whether normalization of ALT levels after combination therapy was associated with reduced incidence of hepatocarcinogenesis because the median follow-up period was 38 months. However, in view of the previous and present results, we consider that maintenance of ALT normalization over a long time after the completion of the combination therapy seems to suppress progression of liver fibrosis and future development of hepatocellular carcinoma. Accordingly, we recommend that even NRs to combination therapy should continue combination therapy for 48 weeks, especially when they achieve ALT normalization during the therapy, to further maintain ALT normalization after the completion of the combination therapy.

In this study, to compare it with past studies, we set the upper limit of ALT to 50 IU/L as a definition of ALT normalization. To review correct ALT normalization, we should lower the upper limit of ALT and study further large-scale populations in the future. And, because we investigated only cases that completed 48 weeks of IFN plus ribavirin treatment, we consider our treatment results about the SVR and the sustained ALT normalization rate were good in comparison with former results. Unfortunately, however, there were cases that had to stop the treatment because of adverse events. Therefore, we consider that even NRs to combination therapy should continue the combination therapy for 48 weeks if they achieve the ALT normalization during the therapy, and then by further maintaining ALT normalization after the completion of the combination therapy, they may suppress the progression of liver fibrosis and prevent future development of hepatocellular carcinoma.

In conclusion, we have demonstrated that combination therapy for patients infected with HCV genotype 1b and a high baseline viral load achieved a high rate of ALT normalization that could be maintained after the

completion of therapy, even in patients who failed to show HCV-RNA eradication. Thus, our results suggest that NRs with ALT normalization should continue the combination therapy to prevent potential future hepatocarcinogenesis.

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