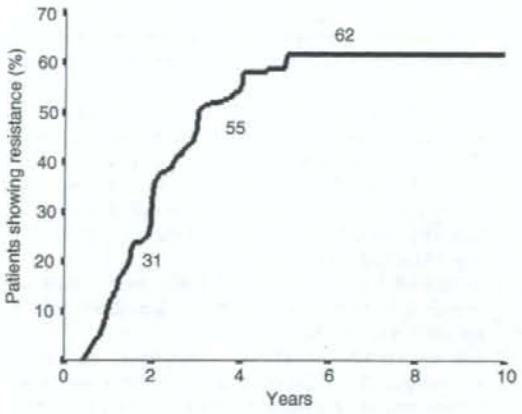

Figure 1 Cumulative percent of patients who exhibited viral resistance during treatment with lamivudine (Kaplan–Meier method).

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,

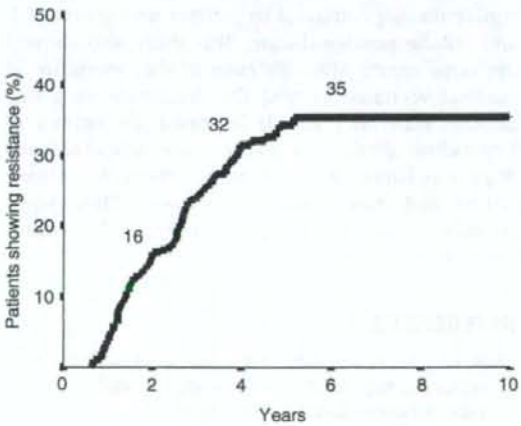

Figure 2 Cumulative percent of patients who developed breakthrough hepatitis during treatment with lamivudine (Kaplan–Meier method).

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

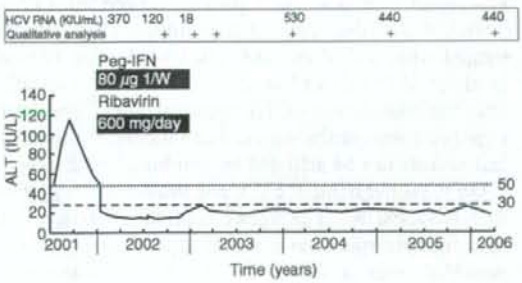

Figure 1 Clinical course of a patient with sustained ALT normalization who showed the persistent presence of HCV-RNA.

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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Original Article

Efficacy and anticarcinogenic activity of interferon for hepatitis C virus-related compensated cirrhosis in patients with genotype 1b low viral load or genotype 2

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Background: We assessed the efficacy and anticarcinogenic effects of interferon (IFN) therapy in patients with hepatitis C virus (HCV)-related cirrhosis.

Methods: The study subjects were 123 Japanese patients with HCV-related cirrhosis with genotype 1b low viral load or genotype 2 who received IFN from 1989 to 2005 (18 patients continue to receive IFN therapy). They included 81 men and 42 women aged 29–74 years (median, 56 years).

Results: Univariate analysis identified four parameters that significantly influenced SVR; viral load (low HCV concentration, $P < 0.001$), duration of IFN therapy (≥ 52 weeks, $P = 0.029$), daily dose of IFN (≥ 6 million units, $P = 0.018$), induction therapy (presence, $P = 0.010$) and choline esterase (> 1.0 ΔpH, $P = 0.037$). Multivariate analysis identified viral load (risk ratio = 6.329, $P < 0.001$) and daily dose of IFN (risk ratio = 2.62, $P = 0.042$) as two independent parameters that influenced SVR. During the observation period, newly developed hepatocellular carcinoma (HCC) was detected in 22 patients. The rates of development of HCC in patients with SVR were 5.8% at the fifth year and 10.3% at the 10th year, compared with 25.8% at the fifth year and 42.5% at the 10th year in non-SVR patients. Multivariate analysis showed that IFN efficacy (SVR) was the only independent factor of hepatocarcinogenesis (hazard ratio: 0.185, 95% confidence interval: 0.042–0.810, $P = 0.025$)

Conclusion: Among patients with HCV-related cirrhosis, the rate of development of HCC is significantly less in patients with SVR.

Key words: cancer prevention, cirrhosis, hepatitis C virus, hepatocellular carcinoma, interferon

INTRODUCTION

HEPATITIS C VIRUS (HCV)-associated hepatocellular carcinoma (HCC) typically develops through a sequence of events that progresses from chronic inflammation through fibrosis and cirrhosis accompanied by dysplasia and ultimately HCC. In a cohort study of Japanese patients with HCV-related cirrhosis,[1] the cumulative appearance rates of HCC at 5, 10 and 15 years were 32.5, 59.6 and 77.4%, respectively. Furthermore, the life expectancy of patients with HCV-related cirrhosis is largely influenced by the development of HCC during the clinical course.[2] Because an effective and curative therapy for HCC remains limited at best, primary prevention of HCC in patients with chronic liver disease is of great importance at present.

Interferon (IFN) is effective in eliminating HCV and reducing the serum level of alanine aminotransferase (ALT) in some patients with chronic hepatitis C.[3–5] The response to IFN therapy is related to various factors such as HCV subtype, serum concentration of HCV, IFN treatment and liver histology.[6–8] Kasahara *et al.* reported that the presence of a persistently normal level of serum aminotransferase after IFN therapy was associated with significant reduction in the incidence of HCC in patients with chronic hepatitis C.[9] Furthermore, reduced incidence of HCC in HCV-associated cirrhosis by IFN has been reported by many investigators;[10–13] in fact, only a few studies have failed to find its benefit.[14,15]

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In order to evaluate whether IFN can reduce the rate of hepatocarcinogenesis and to analyze the pretreatment predictive factors associated with response to IFN and carcinogenesis in patients with HCV-related cirrhosis, we retrospectively analyzed 123 patients with HCV-related cirrhosis.

PATIENTS AND METHODS

Study population

A TOTAL OF 634 patients were diagnosed with HCV-related cirrhosis from 1989 to 2005 at the Department of Hepatology at Toranomon Hospital, Tokyo, Japan. Of these, 267 (42.1%) patients were treated with IFN. They included 140 patients with genotype 1b-high HCV concentration, 24 patients with genotype 1b-low HCV concentration, 38 patients with genotype 2a-high HCV concentration, 42 patients with genotype 2a-low HCV concentration, 18 patients with genotype 2b-high HCV concentration, and one patient with genotype 2b-low HCV concentration. A total of 123 patients with HCV-related cirrhosis with genotype 1b low viral load or genotype 2 were enrolled in this analysis, whose initial sera tested negative for hepatitis B surface antigen by radioimmunoassay (Ausria; Dainabot, Tokyo, Japan) and positive for anti-HCV by the second or third-generation enzyme-linked immunosorbent assay (Dainabot). They included 81 men and 42 women aged 29–74 years (median, 56 years). The diagnosis of liver cirrhosis was based on clinical features, laboratory tests, and peritoneoscopy or liver biopsy. In order to investigate hepatocarcinogenesis in HCV-related cirrhosis, patients coinfected with HBV were excluded. Our institution does not require informed consent for retrospective studies.

Background and laboratory findings

Table 1 shows demographic profiles and results of laboratory tests for the 123 patients at baseline (before treatment with IFN). Quantitative analysis of HCV-RNA was performed using a branched DNA probe assay (bDNA probe assay, version 2.0; Chiron, Dai-ichi Kagaku, Tokyo) and polymerase chain reaction (PCR)-based assay using the protocol provided by the manufacturer (Amplicor HCV Monitor assay version 2.0; Roche Diagnostics, Tokyo, Japan). HCV genotype was classified by PCR, using a mixture of primers for six subtypes known to exist in Japan, as reported previously.[16]

Table 1 Demographics and baseline characteristics of 105 patients in the present study

Age (years)	56 (29–74)†
Sex (M/F)	81/42
BMI (kg/m^2)	23.9 (16.9–35.7)†
Albumin (g/dL)	3.7 (1.8–4.7)†
AST (IU/L)	70 (26–338)†
ALT (IU/L)	80 (11–434)†
Cholesterol (mg/dL)	159 (93–272)†
Choline esterase (ΔpH)	0.8 (0.3–1.5)†
AFP (μg/L)	11 (2–631)†
Ferritin (μg/L)	178 (<10–2076)†
Hyaluronic acid (μl/L)	184 (30–1000)†
FBS (mg/mL)	94 (65–338)†
Platelet (×10^4/μL)	9.8 (2.5–22.3)†
HCV genotype	
1b	24
2a	80
2b	19
HCV-RNA	
High viral load	56
Low viral load	67

†Data expressed as median (range).
High viral load > 100 KIU/mL or > 1 Meq/mL; low viral load < 100 KIU/mL or < 1 Meq/mL.
AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, fasting blood sugar; HCV, hepatitis C virus.

IFN treatment and evaluation of response to therapy

Among the 123 patients, 85 (69.1%) received IFN for the first time, while the remaining 38 (30.9%) patients had received IFN prior to this protocol. In the present study, IFN treatment involved the use of natural or recombinant IFN-α ($n = 83$), natural IFN-β ($n = 38$), or both ($n = 2$). The dosage of IFN varied in this study; 22 (17.9%) patients received 3–9 million units (MU) IFN daily for 4–8 weeks; 38 (30.9%) patients received 3–9 MU IFN daily for 2–8 weeks followed by two or three times per week; 57 (46.3%) patients received intermittent IFN two to three times per week; six patients received pegylated IFN. Among the 123 patients, 10 (8.1%) were treated with both IFN and ribavirin. The median dose of IFN was 399 MU (18–14 778 MU) during a median period of 25 weeks (1.9–602 weeks), and the daily dose was < 6 MU ($n = 59$) and ≥ 6 MU ($n = 58$). In this study, the initial daily administration of IFN for two or more weeks was defined as induction therapy.

The response to IFN was evaluated by clearance of HCV-RNA from serum and serum levels of ALT. Sustained virological response (SVR) was defined as persistent disappearance of HCV-RNA after therapy, while biochemical response (BR) was defined as normalization of ALT levels without elimination of HCV-RNA for at least 6 months after therapy. No response (NR) was defined as elevation or a transient decrease in serum ALT levels with persistent HCV-RNA levels in the serum.

Follow up of patients and diagnosis of HCC

Patients were followed up monthly after diagnosis of liver cirrhosis in our outpatient clinic and monitored clinically by hematological, biochemical and virological tests. In addition to admission to receive IFN treatment, biweekly or monthly follow up was performed in almost all patients who received IFN. Imaging studies were conducted every 3 months in the majority of patients using ultrasonography or computed tomography (CT). Angiography was considered only when HCC was suspected on ultrasonography or CT. The diagnosis of HCC was made by characteristic hypervascular stain on hepatic angiography. When the hepatic nodule did not show hypervascular stain, a fine-needle biopsy was carried out to exclude or diagnose HCC.

Statistical analysis

We used univariate and multivariate logistic regression analyses to determine those factors that contributed to SVR. We also calculated the odds ratios and 95% confidence intervals (95% CI). All *P*-values of less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.15$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. Potential predictive factors associated with SVR included the following 20 variables: age, sex, body mass index (BMI), serum albumin, cholinesterase, total cholesterol, platelet count, α-fetoprotein (AFP), indocyanine green retention rate at 15 min (ICG R15), fasting blood glucose, aspartate aminotransferase (AST), ALT, level of viremia, genotype, combination therapy with ribavirin, duration of IFN therapy, total dose of IFN, daily dose of IFN, method of IFN administration and type of IFN. The incidence of hepatocarcinogenesis was calculated by the Kaplan–Meier method; it was based on the duration between the start of IFN therapy and detection of HCC. Differences in slopes of carcinogenesis curves were evaluated by the log-rank test. Independent factors associated with the development of HCC were studied using stepwise Cox regression analysis. The following 21 variables were analyzed: age, sex, BMI, serum albumin, cholinesterase, total cholesterol, platelet count, AFP, ICG R15, fasting blood glucose, AST, ALT, level of viremia, genotype, combination therapy with ribavirin, duration of IFN therapy, total dose of IFN, daily dose of IFN, method of IFN administration, type of IFN and the effect of IFN (SVR). Statistical analysis was conducted by using SPSS software (version 10; SPSS, Chicago, IL, USA).

RESULTS

Response to IFN

AMONG 123 PATIENTS who received IFN therapy, the response to IFN therapy could be evaluated in 105 who completed the treatment protocol until December 2005, while the remaining 18 patients continue to receive IFN therapy. The dose of IFN was reduced from 6 to 3 MU per day in 20 patients. In 14 of the 20 patients, the IFN dose was reduced according to the study protocol; it was reduced at 1 week after starting IFN in 10 patients and at two weeks in four patients. In the other six patients, the dose was reduced due to thrombocytopenia. Among the 105 patients, 48 (45.7%) showed SVR, 14 (13.3%) showed BR and 43 (41%) were NR.

Efficacy of IFN treatment according to baseline viral load and genotype

For this part of the study, pretreatment viral load was measured in 123 cases and subjects were divided into two groups using a cut-off viral load of 1 Meq/mL or 10^6 copies/mL. Table 2 shows the treatment efficacy estimated by baseline (pretreatment) viral load and genotype among the 105 cases. Of 21 patients with genotype 1b (low HCV concentration), nine (42.9%) showed SVR. Among 31 patients with genotype 2a (high HCV concentration), eight (25.8%) showed SVR and six (19.4%) showed BR. Among 35 patients with genotype 2a (low HCV concentration), 26 (74.3%) showed SVR and two (5.7%) showed BR. Among 17 patients with genotype 2b (high HCV concentration), four (23.5%) showed SVR and two (11.8%) showed BR. The single patient with genotype 2b (low HCV concentration) showed SVR. In summary, among 57 patients with a low viremia level, 36 (63.2%) achieved SVR and six (10.9%) achieved BR, while of 48 patients with a high viremia level, 12 (25.0%) showed SVR and eight (16.7%) patients showed BR.

Table 2 Proportion of patients with SVR among the 105 patients who were treated with IFN for HCV-related cirrhosis

	HCV genotype			Total
	1b	2a	2b	
HCV-RNA high viral load	–	8/31 (25.8)	4/17 (23.5)	12/48 (25.0)
HCV-RNA low viral load	9/21 (42.9)	26/35 (74.3)	1/1 (100.0)	36/57 (63.2)
Total	8/20 (42.9)	34/66 (51.5)	5/18 (27.8)	48/105 (48.0)

Numbers in parentheses are percentages of patients.
High viral load > 100 KIU/mL or > 1 Meq/mL; low viral load < 100 KIU/mL or < 1 Meq/mL.
HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.

Side-effects

Almost all patients treated with IFN showed a variable degree of fever, chills, myalgia, headache, and general malaise after the first injection of IFN. Most patients developed a variable degree of leukocytopenia and thrombocytopenia. IFN therapy was discontinued due to anemia in one patient, thrombocytopenia in one patient, skin eruption in one patient, worsening of diabetes mellitus in one patient, retinopathy in one patient, bleeding from the ocular fundus in one patient, and interstitial pneumonia in one patient (total seven patients, 5.7%).

Predictive factors associated with SVR in multivariate analysis

We then analyzed the data for the entire population sample to determine those factors that could predict SVR. Univariate analysis identified four parameters that significantly influenced the SVR. These included viral load (low HCV concentration, $P < 0.001$), daily dose of IFN (≥ 6 MU, $P = 0.018$), induction therapy (present, $P = 0.010$) and choline esterase level (> 1.0 ΔpH, $P = 0.037$). AFP (< 20 μg/L, $P = 0.058$) and duration of IFN therapy (≥ 52 weeks, $P = 0.064$) were marginally associated with SVR (Table 3). Multivariate analysis identified two parameters that independently influenced SVR, including viral load (risk ratio = 6.99, $P < 0.001$) and daily dose of IFN (risk ratio = 2.62, $P = 0.042$) (Table 4).

Crude rates of hepatocarcinogenesis

Four of the 123 patients received IFN therapy after removal of HCC by either surgical resection or locoregional ablation. Therefore, these four patients were excluded from the following analysis. During the observation period (median: 4.6 years, range: 0.3–14.0 years), HCC developed in 22 (18.5%) of the 119 patients. Of these, three patients showed SVR, 16 patients showed NR and the remaining three patients developed HCC while still receiving IFN therapy and their ALT were below the upper limit of normal. One patient continued IFN therapy after the diagnosis of HCC. The crude rates of hepatocarcinogenesis were 16.8% at the fifth year, 29.1% at the 10th year and 34.2% at the 15th year. The rates of hepatocarcinogenesis in patients with SVR were 5.8% at the fifth year, and 10.3% at the 10th year, and in patients with non-SVR were 25.8% at the fifth year, and 42.5% at the 10th year (Fig. 1). Hepatocarcinogenesis was significantly less frequent in patients with SVR than in patients with non-SVR. (log-rank test, $P = 0.007$).

Predictive factors of hepatocarcinogenesis

Univariate analysis identified three factors that correlated significantly with hepatocarcinogenesis (Table 5). They were the response to IFN therapy (SVR, $P = 0.007$), serum albumin level (> 4.0 g/dL, $P = 0.043$) and choline esterase (> 1.0 ΔpH, $P = 0.009$). Age (> 56 years, $P = 0.080$) and daily dose of IFN (> 6 MU, $P = 0.100$) were marginally associated with hepatocarcinogenesis. Multivariate analysis showed that efficacy of IFN therapy independently influenced the development of HCC in the cohort; SVR was associated with a significant decrease in risk of hepatocarcinogenesis (hazard ratio: 0.185, 95%CI: 0.042–0.810), compared with non-SVR (Table 6).

DISCUSSION

PROGNOSIS OF PATIENTS with HCV-related cirrhosis is greatly affected by the development of HCC, especially during the compensation period.[2] Kasahara *et al.* reported previously that the development of HCC could be suppressed by IFN therapy and elimination of HCV in patients with chronic hepatitis C.[9] Likewise, hepatocarcinogenesis is significantly inhibited by IFN

Table 3 Results of univariate analysis for SVR to IFN therapy in patients with HCV-related cirrhosis

Factor	Category	SVR ($n = 48$)	Non-SVR ($n = 58$)	*P*-value
Age	> 56 years	29 (60.4%)	32 (55.2%)	NS
Sex	Male	31 (64.6%)	38 (65.5%)	NS
BMI	≥ 25 kg/m^2	14 (29.2%)	17 (29.3%)	NS
Albumin	> 4.0 g/dL	17 (35.4%)	18 (31.0%)	NS
AST	> 76 IU/l	23 (47.9%)	29 (50.0%)	NS
ALT	> 100 IU/l	14 (29.2%)	22 (37.9%)	NS
Cholesterol	> 160 mg/dL	26 (54.2%)	27 (46.6%)	NS
Choline esterase	> 1.0 ΔpH	22 (46.8%)	14 (25.5%)	0.037
AFP	> 20 μg/L	11 (23.9%)	24 (43.6%)	0.058
FBS	> 126 mg/mL	10 (23.3%)	6 (11.1%)	NS
Platelets	> 10 × 10^4/mL	27 (56.3%)	27 (46.6%)	NS
HCV genotype	1b	9 (18.8%)	12 (20.7%)	NS
HCV-RNA level	High	12 (25.0%)	36 (62.1%)	< 0.001
Total dose of IFN†	≥ 400 MU	21 (45.7%)	26 (44.8%)	NS
Daily dose of IFN†	≥ 6 MU	31 (67.4%)	25 (43.1%)	0.018
Duration of IFN	≥ 52 weeks	4 (8.3%)	13 (22.4%)	0.064
Induction therapy†	Yes	31 (67.4%)	24 (41.4%)	0.010
Type of IFN	Alpha	29 (60.4%)	38 (65.5%)	NS
Combination with ribavirin	Yes	5 (10.4%)	2 (3.4%)	NS

†Patients who were treated with peglated IFN were excluded from analysis.
High viral load ≥ 100 KIU/mL or ≥ 1 Meq/mL.
AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, fasting blood sugar; HCV, hepatitis C virus; IFN, interferon; NS, not significant; SVR, sustained virological response.

therapy in patients with HCV-related cirrhosis.[10–13] In addition, the incidence of hepatocarcinogenesis is reduced in patients with SVR to IFN.[17]

Previous studies of patients with chronic hepatitis C indicated that HCV genotype and HCV-RNA level are the most significant factors that contribute to SVR and it is assumed that patients with genotype 1b and high viral load respond poorly to IFN therapy.[7] In addition, IFN is less effective in patients with advanced fibrosis than those without.[7,18] In the present study, we retrospectively reviewed the efficacy of IFN therapy and subsequent cancer prevention effect among patients with HCV-related compensated cirrhosis. Patients with both HCV genotype 1b and high viral load were excluded because the rate of HCV elimination among these patients was extremely low and the anticancer effect of such therapy was expected to be low.

In our study, even in patients in whom fibrosis progressed to liver cirrhosis, the SVR ratio was 42.9% with genotype 1b-low HCV concentration, 25.8% with genotype 2a-high HCV concentration, 74.3% with genotype 2a-low HCV concentration, and 23.5% with genotype 2b-high HCV concentration. Thus, the therapeutic efficacy of IFN was approximately equal for liver cirrhosis and chronic hepatitis. Univariate analysis identified four parameters that significantly influenced the SVR. These

Table 4 Results of multivariate analysis for SVR to IFN for HCV-related cirrhosis

Factors	Category	Risk ratio (95% CI)	*P*-value
HCV-RNA level	1: low viral load	6.99 (2.72–17.9)	< 0.001
	2: high viral load	1	
Daily dose of IFN	1: ≥ 6 MU	2.62 (1.04–6.67)	0.042
	2: < 6 MU	1	

High viral load > 100 KIU/mL or > 1 Meq/mL; low viral load < 100 KIU/mL or < 1 Meq/mL.
CI, confidence interval; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.

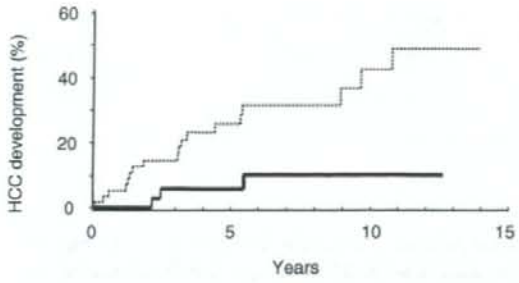

Figure 1 Cumulative rate of development of hepatocellular carcinoma (HCC) in patients treated with interferon. The rates of development of HCC in sustained virological response (SVR) patients at 5 years (5.8%) and 10 years (10.3%) were significantly lower than the respective rates in non-SVR patients (25.8%, 42.5%, $P = 0.007$). —, SVR; ·····, non-SVR.

were viral load, daily dose of IFN, presence of induction therapy and choline esterase.

According to a previous study, the SVR rate was higher among patients who received long-term and high daily dose of IFN.[13] Although the duration of IFN therapy was marginally longer in that study compared to the present study, the results were almost similar to those reported here. In addition, as the advantage of induction therapy among patients with chronic hepatitis has been emphasized in Japan, our result indicates that induction therapy is also important for cirrhotic patients. While the total dose of IFN was also reported previously to correlate with SVR,[19] no significant effect for the dose was recognized in our study. Because the design of our study was not prospective, the treatment schedule varied among patients, and therefore many biases may exist in our results. Further prospective studies are needed to confirm our findings. Multivariate analysis showed that viral load (< 100 KIU/mL) independently influenced SVR, and the SVR rate was as high as 63.2% in those patients with low viral load. As patients with genotype 1b-high viral load were not included in our study, HCV genotype was not significant. When we compared only patients with low viral load, 9/21 (42.9%) patients with genotype 1b, 26/35 patients (74.3%) with genotype 2a, and one patient with genotype 2b showed SVR. Thus, the therapeutic effect of IFN in patients with genotype 2 was significantly high ($P = 0.023$).

Table 5 Results of univariate analysis for development of hepatocellular carcinoma after IFN therapy

Factor	Category	HCC (+) $n = 22$	HCC (-) $n = 97$	*P*-value
Age	> 56 years	16 (72.7%)	50 (51.5%)	0.080
Sex	Male	16 (72.7%)	63 (64.9%)	NS
BMI	≥ 25 kg/m^2	4 (18.2%)	32 (33.0%)	NS
Albumin	> 4.0 g/dL	3 (13.6%)	49 (50.5%)	0.043
AST	> 76 IU/L	11 (50.0%)	47 (48.5%)	NS
ALT	> 100 IU/L	11 (50.0%)	44 (45.4%)	NS
Cholesterol	> 160 mg/dL	13 (59.1%)	49 (50.5%)	NS
Choline esterase	> 1.0 ΔpH	3 (14.3%)	40 (42.1%)	0.009
AFP	> 20 μg/L	9 (42.9%)	31 (32.6%)	NS
FBS	> 126 mg/mL	2 (9.5%)	13 (14.4%)	NS
Platelets	> 10 × 10^4/mL	11 (50.0%)	47 (48.5%)	NS
HCV genotype	1b	16 (72.7%)	81 (83.5%)	NS
HCV-RNA level	High	10 (45.5%)	51 (52.6%)	NS
IFN efficacy	SVR	3 (14.3%)	43 (52.4%)	0.007
Total dose of IFN	≥ 400 MU	10 (45.5%)	44 (48.9%)	NS
Daily dose of IFN	> 6 MU	8 (36.4%)	48 (52.7%)	0.100
Duration of IFN	≥ 52 weeks	7 (31.9%)	22 (22.7%)	NS
Induction therapy	Yes	8 (36.4%)	50 (54.9%)	NS
Type of IFN	Alpha	15 (68.2%)	65 (67.0%)	NS
Combination with ribavirin	Yes	0 (0.0%)	10 (10.3%)	NS

High viral load ≥ 100 KIU/mL or ≥ 1 Meq/mL.
AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, fasting blood sugar; HCV, hepatitis C virus; IFN, interferon; NS, not significant; SVR, sustained virological response.

Table 6 Results of multivariate analysis for development of hepatocellular carcinoma after IFN therapy

Factor	Category	Hazard ratio (95% CI)	*P*-value
IFN efficacy	1: SVR	0.185 (0.042–0.810)	0.025
	2: non-SVR	1	

CI, confidence interval; IFN, interferon; SVR, sustained viral response.

Our results showed that the incidence of HCC increased in a time-dependent manner after induction of IFN (16.8% at the fifth year, 29.1% at the 10th year, and 34.2% at the 15th year). Compared with the rates in Child A cirrhosis patients in our institution who had not been treated with IFN (32.5, 59.6 and 77.4%, respectively),[1] the rate of hepatocarcinogenesis after induction of IFN was half. These results suggest that hepatocarcinogenesis is significantly reduced in patients treated with IFN.

Our retrospective analysis of 123 patients with HCV-related cirrhosis who received IFN treatment showed that the outcome of IFN therapy was significantly associated with carcinogenesis. SVR was associated with a significantly reduced risk of carcinogenesis (hazard ratio: 0.185) compared with non-SVR. Three patients with SVR developed HCC; Case 1 with genotype 2a-low viral load showed SVR after 6 weeks of 3MU IFN-β therapy, but developed HCC 28 months later. Case 2 with genotype 2a-low viral load showed SVR after 6 weeks of 3MU IFN-β therapy but developed HCC 5 years later. Case 3 was also genotype 2a-low viral load who developed HCC at the end of 24 months of 6 MU IFN-α three times per week therapy. According to our previous study, the tumor-doubling time of small HCC ranges from 227 to 607 days (median, 392).[20] The tumor diameter at the time of diagnosis of HCC was 20 mm in Case 1, 26 mm in Case 2 and 10 mm in Case 3. Therefore, it is not clear whether hepatocarcinogenesis developed before or after eradication of HCV.

There was no relationship between the duration of IFN therapy and the rate of hepatocarcinogenesis. Likewise, the duration of IFN therapy and the total dose of IFN did not correlate with hepatocarcinogenesis. It should be noted that 38 patients had been treated with IFN prior to the present study and, thus, we cannot exclude the influence of previous IFN therapy on the development of HCC. Unfortunately, we could not collect data on the type, dose or duration of IFN for these patients because such treatment was carried out in other hospitals. To compensate for this, we analyzed those 85 patients who received IFN for the first time. In these patients, neither the duration of IFN therapy nor the total dose of IFN was significantly associated with the development of HCC.

Our results indicated that long-term IFN therapy in non-SVR patients does not seem to improve long-term prognosis, such as the development of HCC and progression to decompensation. We reported previously that the rate of hepatocarcinogenesis was approximately equal in non-responders and untreated patients with genotype 1b-high virus load.[21] However, the rate was lower in BR cases who were maintained on long-term IFN therapy.[21] In three patients who developed HCC during IFN therapy in the present study, ALT normalization was noted after giving twice or thrice per week IFN-α (3MU), a therapeutic course aimed at reducing the risk of hepatocarcinogenesis. Although the duration of IFN therapy was 3.3, 4.2 and 5.3 years and serum ALT was almost below the upper limit of normal, HCC appeared in these patients during the course of IFN therapy. It is difficult to conclude from the present study that SVR is the only factor that reduced the risk of hepatocarcinogenesis. Although the result of statistical analysis was not significant in our patients, hepatocarcinogenesis was less frequent in patients with BR than in those with NR (the rate of HCC development at 5 years was 17.5% *vs* 29.4%, respectively, data not shown). A larger study is needed to determine the impact of BR on hepatocarcinogenesis among non-SVR patients.

In the present study, only seven of 123 patients (5.7%) discontinued IFN therapy. None developed life-threatening serious adverse effects. This outcome is probably similar to that reported in chronic hepatitis.[21] However, careful follow up is necessary in such cirrhotic patients including liver function tests, platelet and leukocyte counts.

In summary, the SVR rate to IFN therapy was 48.0% in patients with HCV-related compensated cirrhosis except for those patients infected with genotype 1b-high virus load. The SVR was especially high (63.2%) among patients with low viral loads. The risks of hepatocarcinogenesis decreased to almost 50% in all patients treated with IFN and to only 20% among patients with SVR.

Therefore, IFN therapy is strongly recommended for patients with compensated cirrhosis with genotype 1b-low viral load or genotype 2.

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Loss of Hepatitis B Surface Antigen From the Serum of Patients With Chronic Hepatitis Treated With Lamivudine

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Although loss of hepatitis B e antigen (HBeAg) from the serum is sought by treatment with lamivudine, clearance of hepatitis B surface antigen (HBsAg) is the eventual goal of any antiviral therapy. In a single hepatology center in the Metropolitan Tokyo, 486 patients with chronic hepatitis B were followed up for longer than 3 years after they started treatment with lamivudine. HBsAg disappeared from the serum in 17 (3.5%). Age ≥50 years and low HBsAg levels (hemagglutination titer $\leq 2^7$) at the start of lamivudine were significantly more frequent in the patients who did than did not lose HBsAg from the serum. Except for these two factors, there were no differences between the two groups of patients in the prevalence of HBeAg and HBV DNA levels at the baseline, as well as the development of YMDD mutants and breakthrough hepatitis during lamivudine treatment. Using multivariate analysis, age ≥50 years at the start of lamivudine was the only factor predicting the loss of HBsAg (hazard ratio: 2.96 [95% confidence interval: 1.14–7.68], $P = 0.028$). By the method of Kaplan–Meier performed on the 486 patients, the loss of HBsAg was estimated to occur in 3% and 13% of patients, respectively, who had received lamivudine therapy for 5 and 10 years. These results indicate that loss of HBsAg occurs in a minority (3.5%) of patients with chronic hepatitis B who receive lamivudine therapy and more frequently in those with lower HBsAg titers and older ages at the start of treatment. ***J. Med. Virol. 79:1472–1477, 2007.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis; hepatitis B virus; hepatitis B surface antigen; hepatitis B e antigen; lamivudine

NTRODUCTION

Over the world, an estimated 400 million people are infected persistently with hepatitis B virus (HBV), which may progress to chronic hepatitis, cirrhosis and hepatocellular carcinoma [Lai et al., 2003]. Many antiviral drugs have been used for preventing the development of liver disease. Among these, lamivudine was approved for clinical use in 1995 and has gained popularity for the treatment of chronic hepatitis B [Lai et al., 1998; Dienstag et al., 1999; Schalm et al., 2000]. The major goal of lamivudine therapy is loss of hepatitis B e antigen (HBeAg) from the serum, because it reflects decreased HBV replication in the liver [Magnius and Espmark, 1972]. HBV mutants resistant to lamivudine, however, elicits in recipients in parallel with the duration of treatment; they have mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the DNA polymerase/reverse transcriptase [Honkoop et al., 1997; Chayama et al., 1998; Liaw et al., 1999]. Although YMDD mutants induce breakthrough hepatitis, they can be treated by rescue treatments with other antiviral drugs [Suzuki et al., 2002; Hosaka et al., 2004], thereby enabling long-term treatment with lamivudine for 7 years or longer [Kumada, 2003; Akuta et al., 2005].

Loss of hepatitis B surface antigen (HBsAg) from the serum is regarded as the eventual goal of antiviral therapy, because it improves long-term clinical outcomes of HBV infection [Fattovich et al., 1998; de Franchis et al., 2003; Lok and McMahon, 2004]. Data

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are lacking, however, on the efficacy of lamivudine in achieving loss of HBsAg from the sera of patients. In a single hepatology center in Metropolitan Tokyo, 486 patients with chronic hepatitis B were followed-up for the loss of HBsAg. Pretreatment factors influencing the loss of HBsAg were evaluated by univariate and multivariate analyses.

MAERIALS AND METHODS

Patients

During 10 years from 1995 to 2004, 486 patients with chronic hepatitis B received 100 mg of lamivudine daily, and had been followed for 3 years or longer at the Department of Hepatology, Toranomon Hospital in Tokyo, Japan. They had a median age of 43 years (range: 18–76 years) and included 399 (82.1%) men. Chronic hepatitis was diagnosed in 385 (79.2%) by liver biopsies performed under laparoscopy and/or ultrasonic imaging, and cirrhosis in the remaining 101 (20.7%) by liver biopsy and/or ultrasonography plus laparoscopic findings. They had a median serum level of HBV DNA of 7.2 log genome equivalents (LGE) per milliliter, and 338 (69.5%) of them were positive for HBeAg. They received lamivudine for the median of 4.8 years (range: 0.1–15.8 years), and were followed up for the median of 5.0 years (3–15) after the treatment had been started. Lamivudine was discontinued in only 95 (19.5%) patients. Reasons for withdrawal were a change to another antiviral drug in 41, the wish of patients in 29, loss of HBeAg accompanied by normalized ALT levels in 19 and side effects in 6. HBV genotypes were A in 10 (2.1%) patients, B in 32 (6.6%), C in 438 (90.1%), F in 2 (0.4%) and not typeable in the remaining 4 (0.8%) patients.

The 502 patients were followed-up for loss of HBsAg, at least 3 years or longer after treatment with lamivudine had been started, and pretreatment factors predictive of the loss were evaluated by univariate and multivariate analyses. The study design conformed to the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of the institution. Every patient gave an informed consent on the purpose of this study.

Markers of HBV Infection

HBsAg and the corresponding antibody (anti-HBs) were determined by hemagglutination (MyCell; Institute of Immunology Co., Ltd., Tokyo, Japan) and HBeAg by enzyme-linked immunosorbent assay (ELISA) (F-HBe; Sysmex, Kobe, Japan). HBV DNA was determined by transcription-mediated amplification (TMA; Chugai Diagnostics, Tokyo, Japan) and the results were expressed in LGE/ml over a range from 3.7 to 8.7. The six major genotypes of HBV (A–F) were determined serologically by ELISA (HBV GENOTYPE EIA; Institute of Immunology) and the PCR-Invader method with genotype-specific probes [Tadokoro et al., 2006]. YMDD mutants were determined by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) after the method of Chayama et al. [1998].

Statistical Analysis

Factors influencing the loss of HBsAg were evaluated by the log-rank test. Independent factors associated with clearance of HBsAg from the serum by lamivudine treatment were analyzed with a stepwise Cox regression analysis. The relationship between loss of HBsAg and duration of lamivudine therapy was assessed by the method of Kaplan–Meier. Analysis of all data was performed with the computer program SPSS software (SPSS, Inc., Chicago, IL).

RESULTS

Comparison of Baseline Characteristics Between Patients Who Did and Did Not Lose HBsAg From the Serum

During follow-ups for 3 years or longer after the start of lamivudine treatment, 17 of the 486 (3.5%) patients lost HBsAg from the serum. All the 17 patients had lost HBV DNA detectable by a semiquantitative method before HBsAg loss, while only two (12%) patients seroconverted to anti-HBs. Table I compares demographical, clinical and virological characteristics between the patients who did and did not lose HBsAg from the serum by univariate analysis. Older age (≥50 years) and lower HBsAg levels (hemagglutination titer $\leq 2^7$) at the start of lamivudine were significantly more frequent in the patients who did than did not lose HBsAg from the serum. Except for these two factors, there were no differences between them in ALT levels, HBeAg, HBV DNA, and genotypes, and emergence of YMDD mutants as well as breakthrough hepatitis during lamivudine treatment. Among the 14 factors listed in Table I, however, only the age ≥50 years at the start of lamivudine therapy was found to increase the chance for HBsAg loss by multivariate analysis (Table II).

Loss of HBsAg From the Serum of the 17 Patients

Figure 1 illustrates loss of HBsAg from the serum of the 17 patients who received treatment with lamivudine. HBsAg was cleared after the withdrawal of lamivudine treatment in three (18%) of them (cases 1–3). In the remaining 14 patients, HBsAg was cleared from the serum during 3.2–10.8 years while they received lamivudine. Lamivudine was withdrawn in one patient (case 4) after he had received it for 3 years, but it was resumed 6 months thereafter. Anti-HBs developed in sera of cases 2 and 6 after they lost HBsAg.

Within 3 years after the start of lamivudine therapy, HBsAg was cleared from the serum more frequently in the patients who did than did not possess HBeAg at the baseline (57% [4/7] vs. 10% [1/10], $P = 0.036$). Genotypes of HBV were B in two, C in 14 and D in the remaining one; none were infected with HBV genotype A.

TABLE I. Univariate Analysis for Factors Influencing the Loss of HBsAg in Patients Treated With Lamivudine

Factors	Category	HBsAg Lost (n = 17)	HBsAg Persisted (n = 469)	Differences (*P*-value)[a]
Age	≥50 years	9 (83%)	121 (26%)	0.017
Gender	Male	16 (94%)	382 (82%)	NS
HBV infection in mother	Positive	6 (32%)	180 (38%)	NS
History of liver disease	Chronic hepatitis	14 (82%)	371 (79%)	NS
ALT (IU/l)	≥60 IU/l	13 (76%)	360 (77%)	NS
Total bilirubin	≥0.7 mg/dl	14 (82%)	292 (62%)	NS
Cholin esterase	≥1.2 ΔpH	7 (41%)	181 (34%)	NS
HBsAg titer (2^N)[b]	$\leq 2^7$	8 (47%)	84 (18%)	0.032
HBeAg	Positive	7 (41%)	240 (51%)	NS
HBV DNA	≥7.1 LGE[c]/ml	8 (47%)	270 (58%)	NS
HBV genotypes	Genotype C	14 (82%)	438 (93%)	NS
Duration of lamivudine	≥4.1 years	13 (76%)	348 (74%)	NS
YMDD mutants	Present	9 (53%)	264 (56%)	NS
Breakthrough hepatitis	Occurred	4 (24%)	158 (34%)	NS

NS, Not significant.
[a]Evaluated by the log-rank test.
[b]Determined by the passive hemagglutination method.
[c]Log genome equivalents.

Time Course of HBsAg Loss During Treatment With Lamivudine

Figure 2 shows the loss of HBsAg in the 486 patients who had been treated with lamivudine by the method of Kaplan–Meier. HBsAg was estimated to be cleared from the serum in 3% and 13% of the patients, respectively, at 5 and 10 years.

DISCUSSION

Conventionally, the therapeutic efficacy of antiviral treatment in patients with chronic liver disease has been evaluated by loss of HBeAg from the serum [Wong et al., 1993]. However, HBeAg reappears in the sera of some patients from whom lamivudine therapy had been withdrawn [Song et al., 2000; Lee et al., 2002; van Nunen et al., 2003]. Hence, loss of HBeAg from the serum is not a reliable indicator of the antiviral response to lamivudine. Loss of HBsAg from the serum, instead, is usually durable and would be the gold standard for valid virological responses to lamivudine treatment. It has been absent or rare in studies on small series of patients treated with lamivudine [Lai et al., 1998; Dienstag et al., 1999].

TABLE II. Multivariate Analysis for Factors Influencing the Loss of HBsAg[a]

Factor	Category	Hazard ratio	Significance
Age at the start of lamivudine	1: ≤49 years	1	
	2: ≥50 years	2.96 (1.14–7.68)[b]	$P = 0.028$

[a]Evaluated by a stepwise Cox regression analysis on factors listed in Table I.
[b]95% confidence interval.

In the present study, HBsAg was lost from the serum, during or after treatment with lamivudine, in 17 of the 487 (3.5%) patients who had been followed up at least 3 years after the start of treatment. Three patients lost HBsAg after lamivudine had been withdrawn; it is not certain whether or not lamivudine influenced HBsAg loss. The observed incidence is much lower than that in the six patients with de novo HBV reactivator, five (83%) of whom cleared HBsAg from the serum and developed anti-HBs after short-term lamivudine treatment [Umeda et al., 2006]. The efficacy of lamivudine would be much different between patients with ongoing HBV infection and those who have resolved infection and in whom HBV is reactivated following immunosuppressive treatments.

Older age and lower HBsAg levels at the start of lamivudine therapy increased significantly the likelihood of the loss of HBsAg from serum. Anti-HBs developed in only two of the 17 (12%) patients who had cleared HBsAg from the serum. It is not certain, therefore, whether HBsAg disappeared from the circulation, or complexed with anti-HBs and escaped detection, in the patients who lost HBsAg from the serum without developing detectable anti-HBs. When lamivudine treatment can be withdrawn is a matter of conjecture. It would be reasonable to stop lamivudine therapy in the patients who seroconverted to anti-HBs. The patients who lost HBsAg but remain negative for anti-HBs, however, would need to receive lamivudine for at least 6 months, and lamivudine therapy may be stopped in those in whom HBsAg remains negative, provided that they are followed for virological markers at regular intervals.

In previous studies, HBsAg was cleared from the serum in none of the 272 patients treated with lamivudine for 48 weeks and followed for an additional