

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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Short Communication

Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy

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Aim: The clinical significance of hepatitis B virus (HBV) core-related antigen (HBcrAg) in predicting the reactivation of hepatitis after halting lamivudine administration was analyzed.

Methods: A total of 34 patients with chronic hepatitis B were enrolled. Lamivudine was administered for at least 6 months before cessation, and reactivation of hepatitis was defined as elevation of alanine aminotransferase levels to more than 80 IU/L within 12 months of cessation.

Results: In total, 20 (59%) patients experienced hepatitis reactivation. Although concentrations of HBV DNA and HBcrAg in serum did not differ between the two groups of patients at the onset of lamivudine administration, HBcrAg serum levels were significantly higher ($P=0.009$) in the reactivation patients (median 4.9, 25–75% range 4.7–5.9 log unit/mL) than the non-reactivation patients (median 3.2, 25–75% range <3.0–4.5 log unit/mL) post-lamivudine

treatment. The concentration of HBV DNA did not differ between the two groups (median <3.7, 25–75% range <3.7–<3.7 log copy/mL in the reactivation group vs. median <3.7, 25–75% range <3.7–<3.7 log copy/mL in the non-reactivation group). Receiver operating characteristic analysis of HBcrAg concentration showed an area under the curve of 0.764 in predicting patients without reactivation of hepatitis.

Conclusion: HBcrAg can be a useful marker to identify patients who are not at risk of reactivation of severe hepatitis after discontinuation of lamivudine administration.

Key words: chronic hepatitis B, hepatitis B virus core-related antigen, hepatitis B virus DNA, hepatitis reactivation, lamivudine

INTRODUCTION

LAMIVUDINE, A NUCLEOSIDE analog that inhibits reverse transcriptase, has been found to inhibit the replication of hepatitis B virus (HBV), reduce hepatitis, and improve histological findings of the liver in long-

term treatment.^{1,2} Furthermore, it has been shown that lamivudine treatment improves the long-term outcome of patients with chronic hepatitis B.^{3,4} However, there are a number of problems with lamivudine therapy, including hepatitis relapse due to the appearance of YMDD mutant viruses and the reactivation of hepatitis after its discontinuation.^{5,6}

During lamivudine administration, the concentration of serum HBV DNA decreases, and usually becomes undetectable to even high sensitivity HBV DNA assays. However, this undetectable level is an inadequate indicator for safely discontinuing lamivudine

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administration as active hepatitis often recurs in patients post-treatment.

Previously, a chemiluminescence enzyme immunoassay (CLEIA) was developed by our laboratory to detect of hepatitis B core-related antigen (HBcrAg).^{7,8} This HBcrAg CLEIA simultaneously measures the serum levels of hepatitis B core (HBc) and e (HBe) antigens using monoclonal antibodies, which recognize common epitopes of these two denatured antigens because both proteins are transcribed from the precore/core gene and their first 149 amino acids are identical.^{9–11} Although this assay reflects the viral load of HBV in a similar manner to HBV DNA assays during disease progression, HBcrAg CLEIA shows characteristics different from HBV DNA assays under lamivudine administration since HBcrAg levels decrease more slowly than HBV DNA after treatment begins.¹² In the present study, we analyzed the clinical significance of the HBcrAg assay in predicting the likelihood of non-reactivation of hepatitis after discontinuing lamivudine administration in HBV treatment.

METHODS

Patients

A TOTAL OF 34 patients with chronic hepatitis B who were treated with lamivudine for at least 6 months were enrolled in the present study. The patients comprised 20 men and 14 women with a median age of 46 years (range 23–65 years), and were selected retrospectively from five medical institutions in Japan (Shinshu University Hospital, Kyoto Prefectural University Hospital, National Nagasaki Medical Center, Toranomon Hospital, and Hiroshima University Hospital). Written informed consent was obtained from each patient.

Of the 27 patients whose HBV genotype was determined, 25 (93%) were genotype C and the remaining two (7%) were genotype B. Serum HBV DNA was detectable in all patients, and HBe antigen was positive in 16 (47%) of the 34 patients before lamivudine administration.

For treatment of HBV infection, daily doses of 100 mg lamivudine were administered for at least 6 months. Lamivudine administration was stopped when alanine aminotransferase (ALT) levels were reduced to 40 IU/L or less in at least three separate tests. Serum samples were taken at several time points during and after lamivudine administration, and patients were seen at least once a month for at least 12 months after cessation of lamivudine. Estimated duration of HBV DNA

level <3.7 log copy/mL before stopping lamivudine was a median 10 months (range 0–29 months).

Reactivation of hepatitis was defined as elevation of ALT to more than 80 IU/L within 12 months of stopping lamivudine treatment.

Serological markers for HBV

Serum hepatitis B surface antigen, HBe antigen, and anti-HBe antibody were measured by commercially available CLEIA kits (Fujirebio, Tokyo, Japan). Six major genotypes (A–F) of HBV are detectable using the method reported by Mizokami *et al.*¹³ in which the surface gene sequence is amplified by polymerase chain reaction (PCR) and analyzed by restriction fragment length polymorphism. Serum concentration of HBV DNA was determined using a transcription mediated amplification (TMA) assay kit (Chugai Diagnostics Science, Tokyo, Japan) which has a quantitative range of 3.7–8.7 log copy/mL.

Serum concentration of HBcrAg was measured using a CLEIA developed by Fujirebio, as described previously.⁷ Briefly, 150 μ L of serum was incubated with 150 μ L of pretreatment solution containing 15% sodium dodecylsulfate at 60°C for 30 min. After incubation, 120 μ L of pretreated specimen was added to a ferrite microparticle solution in an assay tube. Ferrite microparticles were coated with monoclonal antibodies (HB44, HB61, HB114) against denatured HBc and HBe antigens. After washing, two other monoclonal antibodies against denatured HBcAg and HBeAg (HB91 and HB110) labeled with alkaline phosphatase were added as secondary antibodies. After further washing, 200 μ L of AMPPD (3-(2'-spiroadamantan)-4-methoxy-4-(3''-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt; Applied Biosystems, Bedford, MA) solution was added as substrate, and the assay tube was incubated for 5 min at 37°C.

From this, the relative chemiluminescence intensity was measured, and HBcrAg concentration was determined by comparison with a standard curve generated using recombinant pro-HBe antigen (amino acids, 10–183 of the precore/core gene product). The HBcrAg concentration was expressed as units/mL (U/mL) and a immunoreactivity of recombinant pro-HBe antigen of 10 fg/mL was defined as 1 U/mL. In the present study, the cutoff value of HBcrAg concentration was set at 3.0 log U/mL.

Statistical analysis

The Mann–Whitney *U*-test was used to analyze quantitative data, and Fisher's exact test was used for

qualitative data. Receiver operating characteristic (ROC) curve analysis was used to analyze cut-off levels of HBcrAg concentration for prospective recurrence of hepatitis. Statistical analyses were performed using the SPSS 14.0 J statistical software package (SPSS, Chicago, IL, USA), and a *P*-value of less than 0.05 was considered to be statistically significant.

RESULTS

TWENTY (59%) OF the 34 patients enrolled in the present study showed reactivation of hepatitis within 12 months after discontinuing lamivudine administration, with 15 (75%) showing reactivation within 6 months. The peak serum ALT levels in the 20 reactivation patients ranged from 103 to 1019 IU/L, with a median of 308 IU/L. After lamivudine cessation, the maximum serum HBV DNA was significantly higher ($P < 0.001$) in the reactivation patients (median 7.8, 25–75% range 7.4–8.1 log copy/mL) than in the non-reactivation patients (median 4.8, 25–75% range 4.1–5.9 log copy/mL).

Table 1 shows a comparison of the clinical backgrounds at the onset and completion of lamivudine administration between the two groups of patients. Although backgrounds were similar between the two

groups just prior to lamivudine administration, HBcrAg levels were significantly higher in the reactivation patients after treatment. Both HBV DNA levels and positive rates of HBe antigen were similarly low between the two groups. The duration of undetectable HBV DNA before stopping lamivudine administration was also similar ($P > 0.2$) between the two groups (reactivation patients, median 11 months, 25–75% range 8–13 months vs. non-reactivation patients, median 6 months, 25–75% range 5–13 months).

In 23 patients who were negative for HBe antigen after treatment, HBcrAg levels were significantly higher ($P = 0.011$) in the reactivation patients ($n = 12$, median 4.8 log U/mL, 25–75% range 4.0–5.0 log U/mL) than in non-reactivation patients ($n = 11$, median 3.0 log U/mL, 25–75% range 2.5–4.4 log U/mL). In contrast, levels were similar ($P > 0.2$) between the two groups in 11 patients who were positive for HBe antigen after treatment (reactivation patients $n = 8$, median 5.9 log U/mL, 25–75% range 5.1–6.1 log U/mL vs. non-reactivation patients $n = 3$, median 5.6 log U/mL, 25–75% range 2.5–8.0 log U/mL).

The ability of HBcrAg concentration to predict non-recurrence of hepatitis was analyzed using a ROC curve (Fig. 1), and the area under the curve was as wide as 0.764. The point at which specificity was 0.8 and sensi-

Table 1 Comparison of clinical characteristics at the onset and cessation of lamivudine administration between patients with and without reactivation of hepatitis

Characteristics	Reactivation of hepatitis		<i>P</i> -value†
	Positive ($n = 20$)	Negative ($n = 14$)	
Demographics			
Age (years)	44 (38–51)	50 (35–59)	NS
Sex (male/female)	13/7	7/7	NS
HBV genotype (B/C)	0/16	2/9	NS
At onset of lamivudine administration			
ALT (IU/mL)	103 (57–234)	211 (76–515)	NS
HBeAg (positive)	12 (60%)	4 (29%)	NS
HBV DNA (log copy/mL)	7.1 (6.1–8.1)	6.0 (5.3–7.4)	NS
HBcrAg (log unit/mL)	6.2 (5.6–7.7)	6.4 (5.0–6.6)	NS
At cessation of lamivudine administration			
Duration of lamivudine (months)	12.7 (10.4–16.3)	10.3 (6.4–17)	NS
ALT (IU/mL)	30 (15–36)	21 (15–24)	NS
HBeAg (positive)	8 (40%)	3 (21%)	NS
HBV DNA (log copy/mL)	<3.7 (<3.7–<3.7)	<3.7 (<3.7–<3.7)	NS
HBcrAg (log unit/mL)	4.9 (4.7–5.9)	3.2 (<3.0–4.5)	0.009

†Analysis of continuous variables performed using Mann-Whitney *U*-test; analysis of dichotomous variables performed using Fisher's exact test. Values shown as median (25–75% range) or *n* (%).

ALT, alanine aminotransferase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NS, not significant.

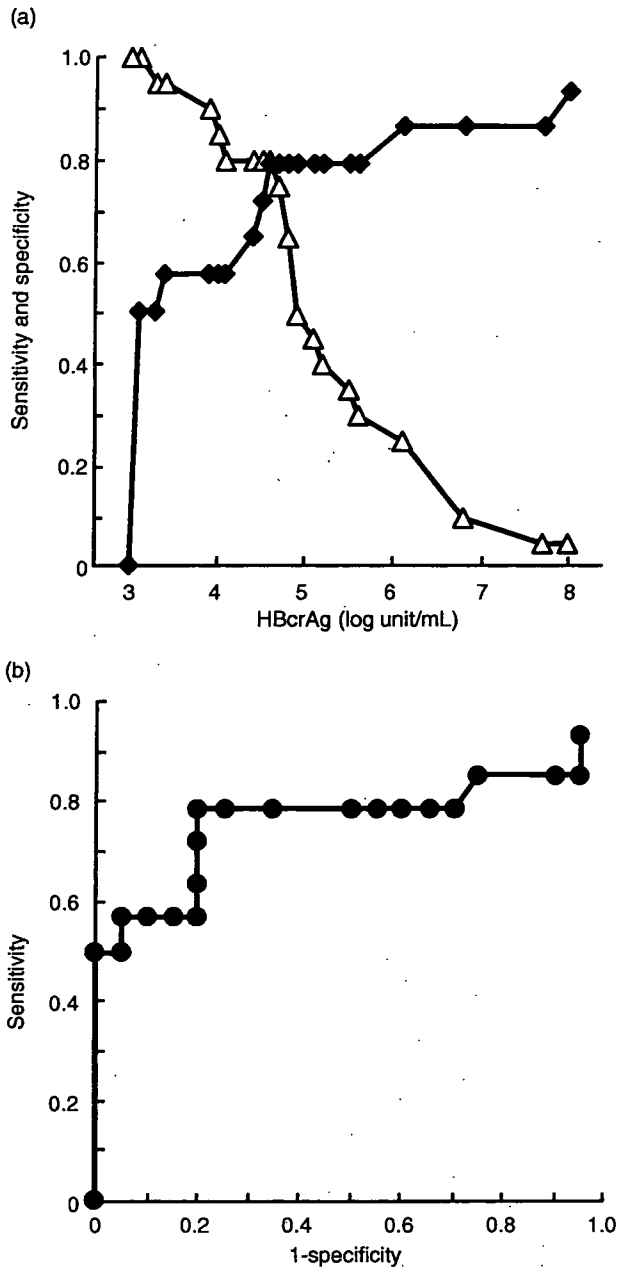


Figure 1 Receiver-operator characteristic (ROC) analysis of hepatitis B core-related antigen (HBcAg) concentration for predicting patients without risk of reactivation of hepatitis within 12 months after halting lamivudine administration. (a) Sensitivity (■) and specificity (Δ) curves according to concentration of HBcAg. (b) The ROC curve with the area under curve of 0.764.

tivity approximately 0.8 was deemed best for halting treatment without the risk of hepatitis recurrence. This point corresponds to an HBcAg concentration of 4.1–4.6 log unit/mL.

DISCUSSION

THE REACTIVATION OF hepatitis following lamivudine administration was defined in the present study as an elevation of serum ALT level to more than 80 IU/L because we sought to find a more reliable indicator for safer discontinuation of lamivudine administration. Under these conditions, the majority (20/34) of patients showed reactivation of hepatitis within 12 months, as has been previously reported.^{5,6} HBV DNA levels at the time of discontinuing lamivudine were similarly low between the two groups of patients, which is understandable as an undetectable reading typically indicates HBV remission following lamivudine therapy. However, HBcAg levels were significantly higher in reactivation patients, implying that HBcAg level is a better marker than HBV DNA level for predicting non-reactivation of hepatitis after discontinuing lamivudine administration especially in patients without HBe antigen.

In this study, ROC curve analyses showed a wide area under the curve of 0.764 in predicting the non-reactivation of HBV with HBcAg level. If the corresponding cutoff is set at 4.5 logU/mL, then both specificity and sensitivity are as high as approximately 0.8. To obtain a higher specificity of 0.9, the cutoff value of HBcAg concentration should be set at 4.0 log unit/mL. In this case, the sensitivity would still be nearly 0.6. The cutoff value of HBcAg for predicting the non-relapse of hepatitis in our study is a little higher than that reported by Shinkai *et al.* (3.4 logU/mL).¹⁴ Because numbers of patients analyzed were small in both studies, further studies are required to confirm the most appropriate cutoff value. It is noteworthy that this cutoff value may also differ among genotypes, which have been reported to be correlated with outcome of chronic HBV infection.¹⁵ However, as over 90% of the patients had genotype C in this study, reactivation could not be analyzed in relation to HBV genotypes.

The HBV is an enveloped DNA virus containing a relaxed circular DNA genome which is converted into a covalently closed circular DNA (cccDNA) episome in the nucleus of infected cells and serves as transcriptional template for the production of viral RNA.^{16,17} Reverse transcription of pregenomic RNA and second-strand DNA synthesis then occur in the cytoplasm within viral

capsids formed by the HBV core protein. Because lamivudine inhibits reverse transcription of pregenomic RNA, it directly suppresses production of HBV virions, and serum HBV DNA levels decrease rapidly after the initiation of lamivudine administration. However, the production of viral proteins is not suppressed by lamivudine as this process does not include reverse transcription. Furthermore, it has been reported that the amount of cccDNA, which also serves as a template for mRNAs, decreases quite slowly after commencement of administration of nucleoside analogs.^{18,19} Thus, it is possible that serum HBcAg levels reflect the cccDNA level in hepatocytes more accurately than serum HBV DNA. High levels of cccDNA are considered to be associated with hepatitis reactivation because they precede reactivation of viral replication and consequent elevation of HBV DNA level in serum.

Lamivudine has already been eliminated from first line therapy in naïve chronic hepatitis B patients due to a higher incidence of developing resistant mutations than new antiviral agents, such as adefovir dipivoxil and entecavir.²⁰ However, the distinct characteristic of the HBcAg assay under lamivudine therapy that is different from other HBV DNA assays is that lamivudine suppresses production of HBV virions by inhibiting reverse transcription of pregenomic RNA, but does not suppress the production of viral proteins, in which reverse transcription is unnecessary. Thus, it is possible that the HBcAg assay may also be useful for patients undergoing entecavir or adefovir dipivoxil administration because the main mechanism of suppressing HBV replication is similar between lamivudine and other antiviral agents. As a considerable number of patients who started lamivudine administration in the past are still taking this treatment now, the present study may be valuable for such patients when they consider changing therapies in the future. Additionally, further studies are required to determine whether the HBcAg assay is indeed applicable to antiviral agents other than lamivudine.

In conclusion, significant markers that can predict reactivation of hepatitis after discontinuing lamivudine administration are clinically valuable because the reactivation of hepatitis is a fundamental problem in lamivudine therapy. Our results suggest that patients with an HBcAg level of less than 4.5 log unit/mL may stop lamivudine administration with a lower risk of reactivation. The present study is a preliminary one because the patients enrolled were selected retrospectively without standardized criteria for stopping lamivudine and the number of patients enrolled was not large; however, the results may be valuable for patients with

hepatitis B undergoing lamivudine therapy as such a diagnostic marker has rarely been reported. Further studies are required to establish the clinical significance of the HBcAg assay in the treatment of hepatitis B.

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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

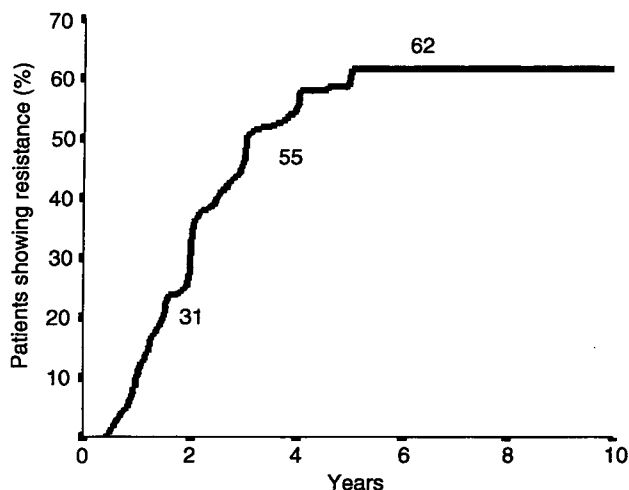


Figure 1 Cumulative percent of patients who exhibited viral resistance during treatment with lamivudine (Kaplan–Meier method).

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,

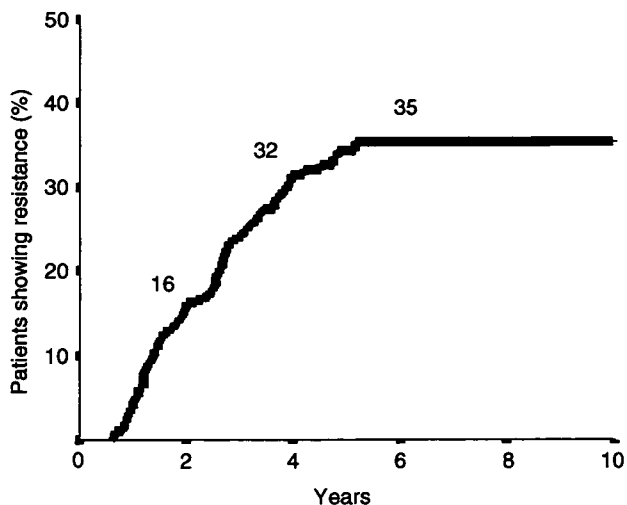


Figure 2 Cumulative percent of patients who developed breakthrough hepatitis during treatment with lamivudine (Kaplan–Meier method).

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 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 study²⁹ 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

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%,

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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 method¹¹); (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 $\geq 100 \times 10^3/\mu\text{l}$; and (viii) signing a consent form of the study protocol that had been approved by Human Ethics Review Committee of Toranomon 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 $\mu\text{g}/\text{kg}/\text{week}$ 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 ($\times 10^3/\mu\text{L}$)	101-309 (177)	101-309 (178)	111-237 (173)	0.550
Fe ($\mu\text{g}/\text{dL}$)	46-308 (139)	46-308 (143)	70-214 (130)	0.393
Ferritin ($\mu\text{g}/\text{L}$)	<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 (<i>n</i> = 9)	7	5	5	5/9
Follow up period; 17-42 (40) months	(77.8%)	(55.6%)	(55.6%)	(55.6%)
NR (<i>n</i> = 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%

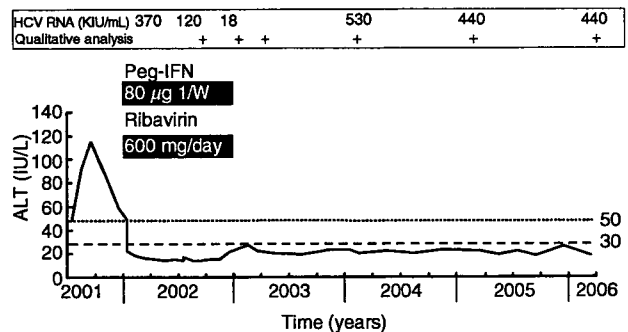
†One patient could not be followed up for 2 years after completion of treatment.

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 ($> 1.0 \Delta\text{pH}$, $P = 0.037$). 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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