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 μL of serum was incubated with 150 μL of pretreatment solution containing 15% sodium dodecylsulfate at 60°C for 30 min. After incubation, 120 μL 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 μL 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		*P*-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.

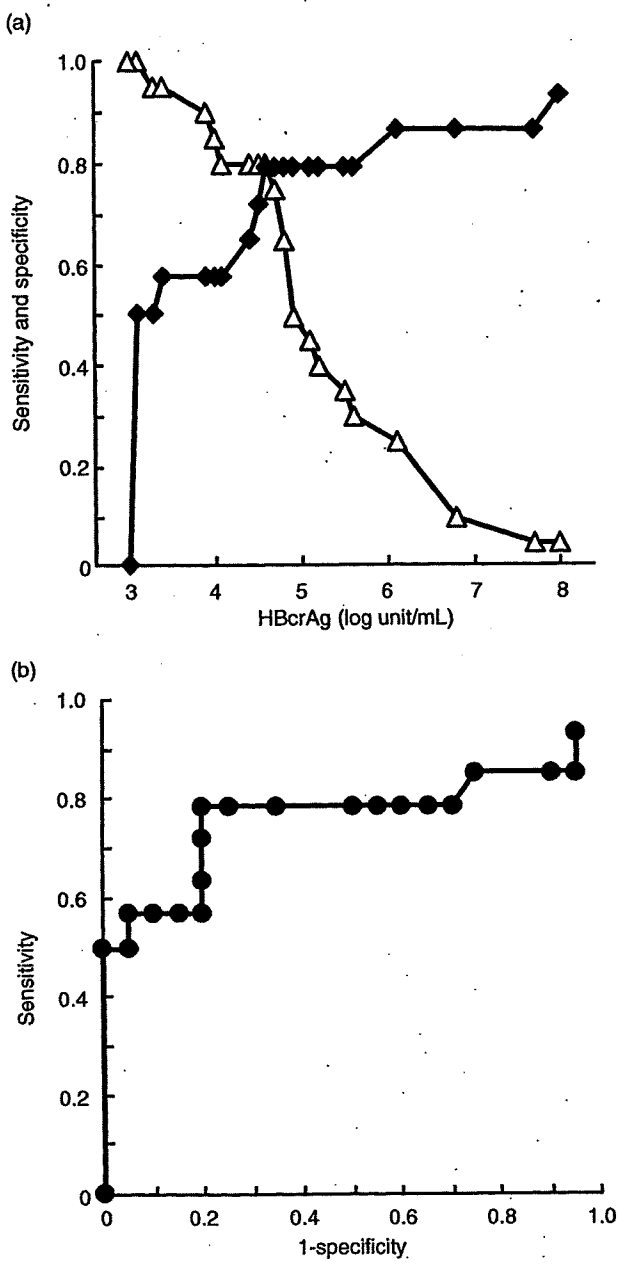

Figure 1 Receiver-operator characteristic (ROC) analysis of hepatitis B core-related antigen (HBcrAg) concentration for predicting patients without risk of reactivation of hepatitis within 12 months after halting lamivudine administration. (a) Sensitivity (■) and specificity (△) curves according to concentration of HBcrAg. (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 HBcrAg 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, HBcrAg levels were significantly higher in reactivation patients, implying that HBcrAg 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 HBcrAg 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 HBcrAg 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 HBcrAg 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.[11,16,17] 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 HBcrAg 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 preceed 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 HBcrAg 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 HBcrAg assay may also 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 HBcrAg 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 HBcrAg 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 HBcrAg assay in the treatment of hepatitis B.

ACKNOWLEDGMENT

THIS RESEARCH WAS supported in part by a Grant-in Aid from Research on hepatitis, the Ministry of Health, Labour and Welfare of Japan.

REFERENCES

1 Dienstag JL, Goldin RD, Heathcote EJ *et al.* Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003; 124: 105–17.
2 Lai CL, Chien RN, Leung NW *et al.* A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; 339: 61–8.
3 Liaw YF, Sung JJ, Chow WC *et al.* Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; 351: 1521–31.
4 Matsumoto A, Tanaka E, Rokuhara A *et al.* Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B. A multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; 32: 173–84.
5 Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. *Hepatology* 1999; 30: 567–72.
6 Lok AS, Lai CL, Leung N *et al.* Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003; 125: 1714–22.
7 Kimura T, Rokuhara A, Sakamoto Y *et al.* Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. *J Clin Microbiol* 2002; 40: 439–45.
8 Rokuhara A, Tanaka E, Matsumoto A *et al.* Clinical evaluation of a new enzyme immunoassay for hepatitis B virus core-related antigen; a marker distinct from viral DNA for monitoring lamivudine treatment. *J Viral Hepat* 2003; 10: 324–30.
9 Bruss V, Gerlich WH. Formation of transmembraneous hepatitis B e-antigen by cotranslational *in vitro* processing of the viral precore protein. *Virology* 1988; 163: 268–75.
10 Garcia PD, Ou JH, Rutter WJ, Walter P. Targeting of the hepatitis B virus precore protein to the endoplasmic reticulum membrane: after signal peptide cleavage translocation can be aborted and the product released into the cytoplasm. *J Cell Biol* 1988; 106: 1093–104.
11 Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997; 337: 1733–45.
12 Tanaka E, Matsumoto A, Suzuki F *et al.* Measurement of hepatitis B virus core-related antigen is valuable for iden-

tifying patients who are at low risk of lamivudine resistance. *Liver Int* 2006; 26: 90–6.
13 Mizokami M, Nakano T, Orito E *et al.* Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. *FEBS Lett* 1999; 450: 66–71.
14 Shinkai N, Tanaka Y, Orito E *et al.* Measurement of hepatitis B virus core-related antigen as predicting factor for relapse after cessation of lamivudine therapy for chronic hepatitis B virus infection. *Hepatol Res* 2006; 36: 272–6.
15 Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? *Hepatology* 2004; 40: 790–2.
16 Mason WS, Halpern MS, England JM *et al.* Experimental transmission of duck hepatitis B virus. *Virology* 1983; 131: 375–84.
17 Tuttleman JS, Pourcel C, Summers J. Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. *Cell* 1986; 47: 451–60.
18 Moraleda G, Saputelli J, Aldrich CE, Averett D, Condreay L, Mason WS. Lack of effect of antiviral therapy in nondividing hepatocyte cultures on the closed circular DNA of woodchuck hepatitis virus. *J Virol* 1997; 71: 9392–9.
19 Werle-Lapostolle B, Bowden S, Locarnini S *et al.* Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology* 2004; 126: 1750–8.
20 Lok ASF, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; 45: 507–39.

Hepatology Research 2007; 37: S42–S46 doi: 10.1111/j.1872-034X.2007.00104.x

Interferon and lamivudine monotherapy on chronic hepatitis B in Japan

Fumitaka Suzuki and Hiromitsu Kumada

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

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

Correspondence: Fumitaka Suzuki, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: fumitakas@toranomon.gr.jp
Accepted 20 July 2006.
Conflict of Interest: none.

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

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

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

INTERFERON THERAPY

Six-month IFN therapy

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

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

Twelve-month IFN therapy

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

LAMIVUDINE THERAPY

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

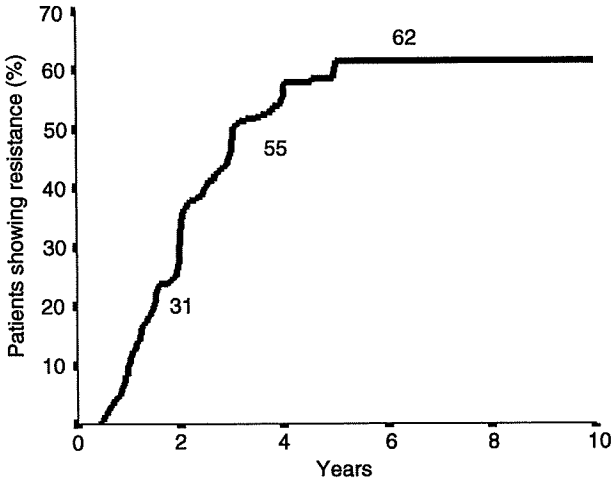

Figure 1 Cumulative percent of patients who exhibited viral resistance during treatment with lamivudine (Kaplan–Meier method).

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

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

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

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

DISCUSSION

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

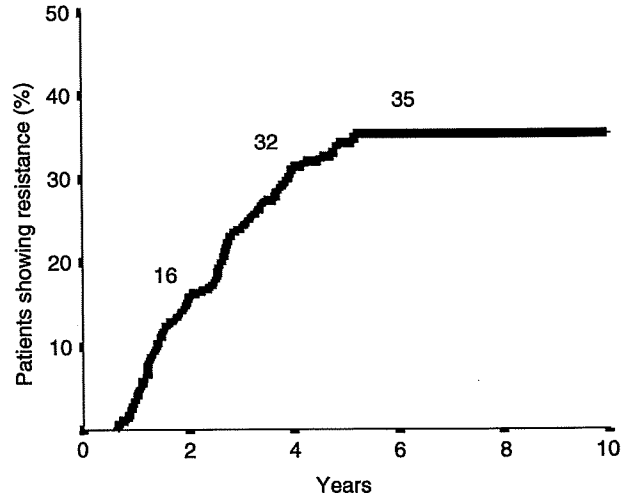

Figure 2 Cumulative percent of patients who developed breakthrough hepatitis during treatment with lamivudine (Kaplan–Meier method).

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

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

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

REFERENCES

1 Beasley RP, Hwang LW, Lin CC *et al.* Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet* 1981; 2: 1129–33.
2 Lok ASF, Heathcote EJ, Hoofnagle JH. Management of hepatitis B 2000-summary of a workshop. *Gastroenterology* 2001; 120: 1828–53.
3 Wong DHK, Cheung AM, O'Rourke K *et al.* Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. *Ann Intern Med* 1993; 119: 312–23.
4 Brook MG, Karayiannis P, Thomas HC. Which patients with chronic hepatitis B virus infection will respond to alpha-interferon therapy? *Hepatology* 1989; 10: 761–3.
5 Perrillo RP, Schiff ER, Davis GL *et al.* A randomized, controlled trial of interferon alfa-2b alone and after prednisolone withdrawal for the treatment of chronic hepatitis B. *N Engl J Med* 1990; 323: 295–301.
6 Perrillo RP, Lai CL, Liaw YF *et al.* Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. *Hepatology* 2002; 36: 186–94.
7 Lok AS, Lai CL, Wu PC *et al.* Long-term follow-up in a randomized controlled trial of recombinant alpha 2-interferon in Chinese patients with chronic hepatitis B infection. *Lancet* 1988; 2: 298–302.
8 Lok AS, Lai CL, Wu PC *et al.* Alpha-interferon treatment in Chinese patients with chronic hepatitis B. *J Hepatol* 1990; 11: 121–5.
9 Yokosuka O, Omata M, Imazeki F *et al.* Changes of hepatitis B virus DNA in liver and serum caused by recombinant leukocyte interferon treatment: analysis of intrahepatic replicative hepatitis B virus DNA. *Hepatology* 1985; 5: 728–34.
10 Dienstag JL, Perrilo RP, Schiff ER *et al.* A preliminary trial of lamivudine for chronic hepatitis B infection. *N Engl J Med* 1995; 333: 1657–61.
11 Lai CL, Chien RN, Leung NW *et al.* A one-year trial of Lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; 339: 61–8.
12 Dienstag JL, Schiff ER, Wright TL *et al.* Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 1999; 341: 1256–63.
13 Suzuki Y, Kumada H, Ikeda K *et al.* Histological changes in liver biopsies after one year of lamivudine treatment in patients with chronic hepatitis B infection. *J Hepatol* 1999; 30: 743–8.
14 Song B-C, Suh DJ, Lee HC *et al.* Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. *Hepatology* 2000; 32: 803–7.
15 Honkoop P, Man RA, Niesters HGM *et al.* Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. *Hepatology* 2000; 32: 635–9.
16 Hoofnagle JH, Di Bisceglie AM. Treatment of chronic viral hepatitis. *N Eng J Med* 1997; 336: 347–56.
17 Leung NWY, Lai CL, Chang TT *et al.* Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. *Hepatology* 2001; 33: 1527–32.
18 Honkoop P, Niesters HG, de Man RA *et al.* Lamivudine resistance in immunocompetent chronic hepatitis B. Incidence and patterns. *J Hepatol* 1997; 26: 1393–5.

19 Chayama K, Suzuki Y, Kobayashi M *et al.* Emergence and takeover of YMDD motif mutant hepatitis B virus during long-term Lamivudine therapy and re-takeover by wild type after cessation of therapy. *Hepatology* 1998; 27: 1711–16.
20 Liaw YF, Leung NW, Chang TT *et al.* Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. *Gastroenterology* 2000; 119: 172–80.
21 Erhardt A, Heineke U, Blondien D *et al.* Mutations of the core promoter and response to interferon treatment in chronic replicative hepatitis B. *Hepatology* 2000; 31: 716–25.
22 Kao JH, Wu NH, Chen PJ *et al.* Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000; 33: 998–1002.
23 Kobayashi M, Arase Y, Ikeda K *et al.* Clinical characteristics of patients infected with hepatitis B virus genotypes A, B and C. *J Gastroenterol* 2002; 37: 35–9.
24 Suzuki F, Arase Y, Akuta N *et al.* Efficacy of 6-month interferon therapy in chronic hepatitis B virus infection in Japan. *J Gastroenterol* 2004; 39: 969–74.
25 Arase Y, Tsubota A, Saitoh S *et al.* Randomized, controlled trial of natural interferon-α therapy for e-antigen-positive chronic hepatitis B patients. *Hepatol Res* 2002; 23: 98–104.
26 Suzuki F, Suzuki Y, Tsubota A *et al.* Mutations of polymerase, precore and core promoter gene in hepatitis B virus during 5-year lamivudine therapy. *J Hepatol* 2002; 37: 824–30.
27 Akuta N, Tsubota A, Suzuki F *et al.* Long-term prognosis by lamivudine monotherapy for severe acute exacerbation in chronic hepatitis B infection: emergence of YMDD motif mutant and risk of breakthrough hepatitis – an open-cohort study. *J Hepatol* 2003; 38: 91–7.
28 Akuta N, Suzuki F, Tsubota A *et al.* The influence of hepatitis B virus genotype on the development of lamivudine resistance during long-term treatment. *J Hepatol* 2003; 38: 315–21.
29 Suzuki F, Tsubota A, Arase Y *et al.* Efficacy of lamivudine therapy and factors associated with emergence of resistance in chronic hepatitis B virus infection in Japan. *Intervirology* 2003; 46: 182–9.
30 Suzuki F, Tsubota A, Arase Y *et al.* HBe antigen loss during lamivudine therapy is not caused by mutations in precore and core promoter genes in patients with chronic hepatitis B. *J Med Virol* 2003; 70: 355–60.
31 Suzuki Y, Arase Y, Ikeda K *et al.* Histological improvements after a three-year lamivudine therapy in patients with chronic hepatitis B in whom YMDD mutants did not or did develop. *Intervirology* 2003; 46: 164–70.
32 Akuta N, Suzuki F, Kobayashi M *et al.* Virological and biochemical relapse according to YMDD motif mutant type during long-term lamivudine monotherapy. *J Med Virol* 2003; 71: 504–510.
33 Tsubota A, Arase Y, Suzuki F *et al.* Severe acute exacerbation of liver disease may reduce or delay emergence of YMDD motif mutants in long-term lamivudine therapy for hepatitis B e antigen-positive chronic hepatitis B. *J Med Virol* 2004; 73: 7–12.
34 Ooga H, Suzuki F, Tsubota A *et al.* Efficacy of lamivudine treatment in Japanese patients with hepatitis B virus-related cirrhosis. *J Gastroenterol* 2004; 39: 1078–84.
35 Tsubota A, Arase Y, Suzuki Y *et al.* Lamivudine monotherapy for spontaneous severe acute exacerbation of chronic hepatitis B. *J Gastroenterol Hepatol* 2005; 20: 426–32.
36 Akuta N, Suzuki F, Suzuki Y *et al.* Favorable efficacy of long-term lamivudine therapy in patients with chronic hepatitis B; an 8-year follow-up study. *J Med Virol* 2005; 75: 491–8.
37 Suzuki F, Akuta N, Suzuki Y *et al.* Clinical and virological features of non-breakthrough and severe exacerbation due to lamivudine-resistant hepatitis B virus mutants. *J Med Virol* 2006; 78: 341–52.
38 Hadziyannis S, Bramou T, Makris A *et al.* Interferon alfa-2b treatment of HBeAg negative/serum HBV DNA positive chronic active hepatitis B. *J Hepatol* 1990; 11: S133–6.
39 Pastore G, Santantonio T, Milella A *et al.* Anti-HBe-positive chronic hepatitis B with HBV-DNA in serum: response to a 6-month course of lymphoblastoid interferon. *J Hepatol* 1992; 20: 221–5.
40 Fattovich G, Farci P, Rugge M *et al.* A randomized, controlled trial of lymphoblastoid interferon-alfa in patients with chronic hepatitis B lacking HBeAg. *Hepatology* 1992; 15: 584–9.
41 Lampertico P, Del Ninno E, Manzin A *et al.* A randomized, controlled trial of a 24-month course of interferon alfa 2b in patients with chronic hepatitis B who had hepatitis B virus DNA without hepatitis B e antigen in serum. *Hepatology* 1997; 26: 1621–5.
42 Arase Y, Tsubota A, Saitoh S *et al.* Randomized, controlled trial of natural interferon-α therapy for e-antigen-positive chronic hepatitis B patients. *Hepatol Res* 2002; 23: 98–104.
43 Manesis E, Savas S, Petraki F *et al.* Interferon treatment and retreatment of HBeAg negative chronic hepatitis B: twelve-year follow up of 209 patients (Abstract). *Hepatology* 1999; 30: 349A.

Hepatology Research 2007; 37: 787–792 doi: 10.1111/j.1872-034X.2007.00132.x

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

Hitomi Sezaki, Fumitaka Suzuki, Yusuke Kawamura, Hiromi Yatsuji, Tetsuya Hosaka, Norio Akuta, Masahiro Kobayashi, Yoshiyuki Suzuki, Yasuji Arase, Kenji Ikeda and Hiromitsu Kumada

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

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]

Correspondence: Dr Hitomi Sezaki, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: hitomis@mx1.harmonix.ne.jp
Received 9 November 2006; revision 27 March 2007; accepted 2 April 2007.

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

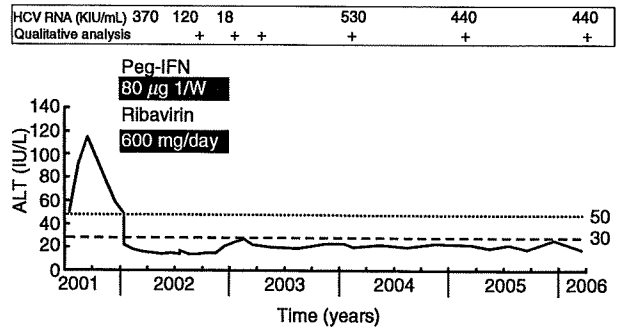

Figure 1 Clinical course of a patient with sustained ALT normalization who showed the persistent presence of HCV-RNA.

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.

REFERENCES

1 Niederau C, Lange S, Heintges T *et al.* Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology* 1998; 28: 1687–95.
2 Ikeda K, Saitoh S, Suzuki Y *et al.* Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* 1998; 28: 930–8.
3 Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. *N Engl J Med* 1999; 340: 1228–33.
4 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; 329: 1124–30.
5 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alpha-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet* 2001; 358: 958–65.
6 McHutchison JG, Gordon SC, Schiff ER *et al.* Interferon alpha-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med* 1998; 339: 1485–92.
7 Poynard T, Marcellin P, Lee SS *et al.* Randomised trial of interferon alpha 2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha 2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *Lancet* 1998; 352: 1426–32.
8 Reichard O, Norkrans G, Frydén A *et al.* Randomised double-blind, placebo-controlled trial of interferon α-2b with and without ribavirin for chronic hepatitis C. *Lancet* 1998; 351: 83–7.
9 Lindsay KL, Trepo C, Heintges T *et al.* A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; 34: 395–403.
10 Iino S, Tomita E, Kumada H *et al.* Impact of daily high-dose IFN α-2b plus ribavirin combination therapy on reduction of ALT levels in patients with chronic hepatitis C with genotype 1 and high HCV-RNA levels. *Hepatol Res* 2005; 31: 88–94.
11 Chayama K, Tsubota A, Arase Y *et al.* Genotypic subtyping of hepatitis C virus. *J Gastroenterol Hepatol* 1993; 8: 150–6.
12 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; 19: 1513–20.
13 Shehab TM, Fontana RJ, Oberhelman K, Marrero JA, Su GL, Lok ASF. Effectiveness of interferon α-2b and ribavirin combination therapy in the treatment of naïve chronic hepatitis C patients in clinical practice. *Clin Gastroenterol Hepatol* 2004; 2: 425–31.
14 Tsubota A, Arase Y, Someya T *et al.* Early viral kinetics and treatment outcome in combination of high-dose interferon induction vs. pegylated interferon plus ribavirin for naïve patients infected with hepatitis C virus of genotype 1b and high viral load. *J Med Virol* 2005; 75: 27–34.
15 Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon α-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; 38: 645–52.
16 Akuta N, Suzuki F, Sezaki H *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; 48: 372–80.
17 Akuta N, Suzuki F, Tsubota A *et al.* Efficacy of interferon monotherapy to 394 consecutive naïve cases infected with hepatitis C virus genotype 2a in Japan: therapy efficacy as consequence of tripartite interferon of viral, host and interferon treatment-related factors. *J Hepatol* 2002; 37: 831–6.
18 Berg T, Sarrazin C, Herrmann E *et al.* Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. *Hepatology* 2003; 37: 600–9.
19 Berg T, Von Wagner M, Nasser S *et al.* Extended treatment duration for hepatitis C virus type 1: Comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. *Gastroenterology* 2006; 130: 1086–97.
20 Iino S. *Incidence of Progression to Hepatocellular Carcinoma Following Interferon Treatment of Chronic Hepatitis C Using a Survey by Questionnaire.* Annual report from Non-A, Non-B Hepatitis Research Group sponsored ministry of Health and Welfare, Tokyo. 1997; 49–52 (in Japanese).
21 Kasahara A, Hayashi N, Mochizuki K *et al.* Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. *Hepatology* 1998; 27: 1394–402.
22 Freeman AJ, Law MG, Kaldor JM, Dore GJ. Predicting progression to cirrhosis in chronic hepatitis C virus infection. *J Viral Hepat* 2003; 10: 285–93.
23 Ikeda K, Arase Y, Saitoh S *et al.* Anticarcinogenic impact of interferon on patients with chronic hepatitis C: a large-scale long-term study in a single center. *Intervirology* 2006; 49: 82–90.
24 Akuta N, Suzuki F, Suzuki Y *et al.* Long-term follow-up of interferon monotherapy in 454 consecutive naïve patients infected with hepatitis C virus: multi-course interferon therapy may reduce the risk of hepatocellular carcinoma and increase survival. *Scand J Gastroenterol* 2005; 40: 688–96.

Hepatology Research 2007; 37: 793–800 doi: 10.1111/j.1872-034X.2007.00140.x

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

Eiko Hasegawa, Masahiro Kobayashi, Yusuke Kawamura, Hiromi Yatsuji, Hitomi Sezaki, Tetsuya Hosaka, Norio Akuta, Fumitaka Suzuki, Yoshiyuki Suzuki, Yasuji Arase, Kenji Ikeda and Hiromitsu Kumada

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

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]

Correspondence: Dr Masahiro Kobayashi, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: mshkobayashi@toranomon.gr.jp
Received 11 December 2006; revision 28 February 2007; accepted 7 April 2007.

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