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Efficacy of 6-month interferon therapy in chronic hepatitis B virus infection in Japan

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Background. In Japan, there are few studies of long-term (more than 1 month) interferon (IFN) therapy for chronic hepatitis B (CHB). In this retrospective study, we investigated the efficacy and predictors of response to 6-month IFN therapy. ***Methods.*** We analyzed 66 Japanese patients with CHB who were treated with IFN for 6 months. They comprised patients who were hepatitis B e antigen (HBeAg)-positive (n = 45) and -negative (n = 21). One (2%), 8 (12%), and 51 (77%) patients were infected with hepatitis B virus (HBV) genotypes A, B, and C, respectively. Responders in patients positive for HBeAg were defined as those who showed normalization of serum alanine aminotransferase (ALT) level, HBeAg loss, and HBV DNA negativity at 6 months after completion of IFN therapy. In patients negative for HBeAg, responders were defined as those patients who showed normalization of ALT level and HBV DNA negativity at the same 6-month time point. ***Results.*** Of the 45 patients with HBeAg at the commencement of IFN therapy, 9 (20%) were responders. Young patients, especially those with a high serum ALT level, were significantly more likely to respond to IFN therapy. Of the 21 patients negative for HBeAg, 13 (62%) were responders. There were no significant differences in clinical characteristics between responders and nonresponders among patients negative for HBeAg. Multivariate analyses identified HBeAg negativity and young age as independent factors associated with a positive response to 6-month IFN therapy. However, long-term follow-up of the treated patients showed a fall in the response rate. ***Conclusions.*** The response rate to 6-month IFN therapy among HBeAg-positive patients was low. However, young patients may require long-term IFN therapy.

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Introduction

Hepatitis B virus (HBV) infection is a common disease that can lead to a chronic carrier state, and it is associated with the 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, whereas long-term therapy with lamivudine may result in drug resistance. A metaanalysis of IFN therapy published in 1993 reviewed 15 randomized controlled studies involving 837 adult patients who received IFN-α at doses of 5–10 million units (MU), administered at intervals ranging from daily to three times weekly, for 4–6 months.[3] Loss of hepatitis B e antigen (HBeAg) occurred in 33% of the treated patients compared with 12% of controls. Loss of detectable HBV DNA and normalization of alanine aminotransferase (ALT) level were also more common in treated than control patients. The major 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, in the 1990s in Japan, the duration of IFN therapy was mainly 1 month, and the efficacy was limited.[7–9]

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.[10] Another study, of 58 Taiwanese patients who received IFN treatment for HBeAg-positive CHB, found that patients with genotype B had

a significantly higher rate of HBeAg loss compared with those with genotype C.[11] Moreover, Sugauchi et al.[12] proposed that genotype B could be provisionally classified into a Ba ("a" for Asia) subgroup and a Bj ("j" for Japan) subgroup, and that such virological differences could explain the clinical differences in various Asian countries. Our previous study indicated that, in Japan, the proportions of HBV infection associated with genotypes B and C were 9% and 88%, respectively.[13] Our study also showed that the majority of genotype B patients were HBeAg-negative at the first examination and showed a mild degree of hepatic fibrosis, while genotype C infection was associated with progressive liver fibrosis.[13] Therefore, mainly patients with genotype C of CHB have received antiviral treatment in Japan.

In Japan, there are few studies of long-term (more than 6 months) IFN therapy for CHB. The present study was designed to re-examine retrospectively the efficacy of 6-month IFN therapy and to determine the potential predictors of a positive response to IFN treatment in Japan.

Table 1. Characteristics of patients at commencement of interferon therapy

Demographic data	
Total number of patients	66
Sex (female/male)	19/47
Age (years)[a]	36 (21–61)
Family history of liver disease	37 (56%)
Previous interferon treatment	13 (20%)
Total dose of interferon (Mega Units)[a]	363 (120–1892)
Duration of treatment (weeks)[a]	24 (23–26)
Laboratory data	
Aspartate aminotransferase (IU/l)[a]	87 (30–755)
Alanine aminotransferase (IU/l)[a]	169 (47–802)
Bilirubin (mg/dl)[a]	0.8 (0.3–1.8)
Albumin (g/dl)[a]	3.9 (3.1–4.8)
Staging of liver history (F1/2/3/4/ND)[b]	37/15/6/2/6
Serum HBV DNA[c] (bDNA; Meq/ml)[a]	41.5 (0.5–4000)
HBeAg-positive	45 (68%)
HBV genotype (A/B/C/unknown)	1/8/51/6

[a] Data valves are medians (ranges)
[b] Scores could range from 0 to 4; a score of 4 indicates liver cirrhosis. ND, not done
[c] HBV DNA levels were measured by branched-chain DNA probe assay (bDNA). All HBV DNA values below the lower limit of detection ($<0.7 \times 10^6$ viral genomic equivalents/ml) were set to 0.5 and those over the upper limite of detection ($>3800 \times 10^6$ viral genomic equivalents/ml genomic equivalents) were set to 4000 for calculation purposes

Patients and methods

Patients

We retrospectively studied 66 Japanese adult patients (19 women and 47 men) who commenced IFN treatment between June 1988 and October 2002 at the Department of Gastroenterology of Toranomon Hospital (Table 1). All patients were followed up from the commencement of therapy at our hospital. They all were positive for hepatitis B surface antigen (HBsAg) in the serum for more than 6 months. Causes of hepatitis other than HBV were excluded, such as infection with hepatitis C virus, as well as autoimmune hepatitis. None of the patients had a history of drug abuse or alcoholic hepatitis, and none had received lamivudine therapy before the commencement of IFN.

Interferon therapy and assessment of response to therapy

Patients received 3 to 12 Mega Units (MU) of IFN-α or -β (Sumiferon; Sumitomo Pharmaceutical, Osaka, Japan; Canferon A; Takeda Chemical Industries, Osaka, Japan; Intron A; Schering-Plough, Osaka, Japan; or Feron; Toray, Tokyo, Japan). The regimen in 36 patients was two or three times a week for 6 months, while that applied for the remaining 30 patients was daily for 4 or 8 weeks, followed by three times a week for 20 or 16 weeks. The duration of treatment was 6 months (23–26 weeks) and the median total dose of IFN was 363 MU (Table 1). In patients with HBeAg, responders were defined as those patients who showed normalization of serum ALT level (normal level, 6–50 IU/l), HBeAg loss, and HBV DNA negativity at 6 months after the completion of IFN therapy. On the other hand, in patients negative for HBeAg, responders were defined as those patients who showed normalization of ALT level and HBV DNA negativity at 6 months after the completion of IFN therapy. All patients except for responders were considered nonresponders.

Blood tests and serum viral markers

Routine biochemical tests were performed, using standard procedures, before and at least once every month during therapy. Serial blood samples were taken from some patients before and during therapy and were stored at −80°C until used for measuring HBV DNA. HBsAg, HBeAg, and anti-HBe were determined by radioimmunoassay kits (Abbot Diagnostics, Chicago, IL, USA). HBV DNA was measured by branched-chain DNA probe assay (bDNA; Chiron Laboratory Service, Van Nuys, CA, USA); the lower limit of detection of this assay is 0.7×10^6 viral genomic equivalents/ml (0.7 Meq/ml).

HBV genotype

The six major genotypes of HBV (A, B, C, D, E, and F) were determined by enzyme-linked immunosorbent assay (ELISA; HBV Genotype EIA, Institute of Immu-

nology, Tokyo, Japan) according to the method described by Usuda et al.[14] This method involves the use of monoclonal antibodies directed against five epitopes, which are exposed on the product of the preS2 region of the HBV genome. Because the expression of the five preS2 epitopes is influenced by the HBV genotype, their combination enables determination of the genotypes serologically. Thus, the genotype was determined as A to F. The validity of this ELISA for serological determination of the five HBV genotypes has been verified previously.[14]

Subgroups Ba and Bj of genotype B were determined by a polymerase chain reaction (PCR) method. For this purpose, DNA was extracted from 100 µl of serum. The first PCR for detection of the precore and core region (nucleotide [nt] 1690 to 2600) of HBV DNA was performed using primers BJF3 (5′-CCGACCTTGAGGC ATACTTC-3′; sense) and BJR4 (5′-GGGTCCCACA AATTGCTTAC-3′; antisense) under conditions of initial denaturation for 4 min, 35 cycles of amplification at 94°C for 1 min, 55°C for 2 min, 72°C for 3 min, and 72°C for 7 min. The second PCR reaction was performed under the same reaction conditions, using primers BJF1 (5′-GCTGTGCCTTGGGTGGCTTTG-3′; sense) and BJR2 (5′-GCGACGCGGTGATTGAGACCT-3′; antisense). The amplified PCR products were purified and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems Japan, Tokyo, Japan). Phylogenetic analysis was performed by the following method. The total numbers of synonymous and nonsynonymous substitutions among nucleotide sequences were estimated by the method of Gojobori et al.[15] Using this number, a phylogenetic tree was constructed by the neighbor-joining method.[16] Genotype B subgroups (Ba and Bj) were determined by these results.

Statistical analysis

Differences between groups were examined for statistical significance using the χ^2 test or Fisher's exact test and the Mann-Whitney *U*-test where appropriate. The above calculations were performed using StatView software (version 4.5J; Abacus Concepts, Berkeley, CA, USA). Independent predictive factors associated with response to IFN treatment were determined using multivariate multiple logistic regression. The following 12 potential predictors of the response to IFN treatment were assessed in this study: age, sex, family history of an HBV carrier, pretreatment with IFN, IFN total dose, method of IFN administration, HBV genotype, severity of liver disease (mild fibrosis [F1] or not [F2–4]), aspartate aminotransferase (AST), ALT, HBeAg, and HBV DNA level. All factors found to be at least marginally associated with response to IFN therapy ($P < 0.15$) were entered into the multivariate multiple logistic regression analysis. Multivariate multiple logistic regression was performed using the Windows SPSS software package (SPSS, Chicago, IL, USA). The odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the relative risk. A two-tailed *P* value of less than 0.05 was considered statistically significant.

Results

Study population

One (2%), 8 (12%), and 51 (77%) patients were infected with HBV genotypes A, B, and C, respectively. The genotype in the remaining 6 patients could not be determined. The baseline characteristics of the patients are shown in Table 1. Although the numbers of patients with genotypes A and B were small, the distribution of HBV genotype was similar to that in patients with chronic hepatitis B who received care in our hospital, with a follow-up period of more than 2 years.[13] The 1 patient with genotype A, 1 of the 8 with genotype B, 37 of the 51 with genotype C, and all 6 with unknown genotype had HBeAg at the commencement of treatment. Seven of the 8 patients with genotype B were Bj and the other was the Ba type. At the commencement of IFN therapy, the ALT levels of 2 patients (both HBeAg-positive) were normal, and the HBV DNA levels of 9 patients (3 who were HBeAg-positive and 6 who were-negative) were less than 0.7 Meq/ml. However, these parameters in the above patients had been elevated within the 3 months before the treatment started.

Response to interferon therapy

Of the 45 patients with HBeAg at the commencement of IFN therapy, 9 (20%) were responders. Table 2 shows the demographic and clinical characteristics of responders and non-responders in these patients with HBeAg. Patients of younger age or higher ALT level had significantly higher rates of antiviral response to IFN than the others. Other characteristics were not related to the response to therapy. The one patient with HBeAg of genotype B (Bj) responded to IFN therapy.

On the other hand, of the 21 patients negative for HBeAg, 13 (62%) were responders. There were no significant differences in the clinical characteristics of responders and non-responders in this group (Table 3). Among the patients negative for HBeAg, the genotype (B or C) did not correlate with response to IFN therapy. The single patient with genotype Ba was a non-responder. Of the 6 patients with genotype Bj, 4 were responders while the remaining 2 were non-responders.

Table 2. Analysis of predictors of response to interferon therapy in patients positive for HBeAg

	Responders (n = 9)	Non-responder (n = 36)	P value
Sex (female/male)	4/5	11/25	0.45
Age (years)[a]	30 (21–35)	39 (23–59)	0.0048
Family history of liver disease	5 (56%)	22 (61%)	1.0
Previous interferon treatment	1 (11%)	8 (22%)	0.66
Total dose of interferon (MU)[a]	408 (120–1892)	408 (120–774)	0.80
Method of interferon administration (ED + I/I)[b]	5/4	21/15	1.0
Staging of liver history (F1/2/3/4/ND)	3/1/1/1/3	21/9/4/0/2	0.11
ALT (IU/l)[a]	263 (126–500)	149 (47–701)	0.049
Serum HBV DNA (bDNA; Meq/ml)[a]	7.9 (0.5–4000)	303 (0.5–4000)	0.18
HBV genotype (A/B/C/unknown)	0/1/7/1	1/0/30/5	0.12

[a] Data values are medians (ranges)
[b] ED + I, initially every day following intermittent therapy; I, only intermittent therapy

Table 3. Analysis of predictors of response to interferon therapy in patients negative for HBeAg

	Responders (n = 13)	Non-responders (n = 8)	P value
Sex (female/male)	3/10	1/7	1.0
Age (years)[a]	42 (30–60)	37 (28–61)	0.54
Family history of liver disease	6 (46%)	4 (50%)	1.0
Previous interferon treatment	3 (23%)	1 (13%)	1.0
Total dose of interferon (MU)[a]	219 (129–624)	152 (120–533)	0.45
Method of interferon administration (ED + I/I)[b]	3/10	1/7	1.0
Staging of liver histology (F1/2/3/4/ND)	6/4/1/1/1	7/1/0/0/0	0.36
ALT (IU/l)[a]	185 (58–712)	153 (54–802)	0.66
Serum HBV DNA (bDNA; Meq/ml)[a]	7.7 (0.5–770)	1 (0.5–47)	0.32
HBV genotype (A/B/C/unknown)	0/4/9/0	0/3/5/0	1.0

[a] Data values are medians (ranges)
[b] ED + I, initially every day following intermittent therapy; I, only intermittent therapy

Two patients with normal ALT levels at commencement were non-responders. Of six HBeAg-negative patients with undetectable levels of HBV DNA at commencement, three were responders. On the other hand, of three HBeAg-positive patients with undetectable levels of HBV DNA at commencement, one patient was a responder.

Long-term outcome after IFN therapy

In this study, the median follow-up period after IFN therapy was 2 years (range, 0.5–6 years). We analyzed long-term outcome in 31 patients positive for HBeAg and 18 patients negative for HBeAg, in whom the follow-up period was 1 year or more. In the 31 HBeAg-positive patients, the number of responders had decreased from 8 (26%) after 6 months to 5 (16%) at 1–5 years. On the other hand, in the 18 HBeAg-negative patients, the number of responders decreased from 11 (61%) after 6 months to 6 (33%) at 1–6 years.

Evaluation of efficacy of IFN in relation to clinical factors

Data for all patients were subjected to univariate analysis to determine the clinical factors that contributed to the efficacy of IFN treatment. In this analysis, the following two factors significantly influenced the response to IFN: HBeAg negativity (OR, 6.5; 95% CI, 2.1–20.4; P = 0.0013) and low HBV DNA level (<100 vs ≧ 100 Meq/ml; OR, 3.4; 95% CI, 1.0–10.8; P = 0.043). Moreover, ALT level (≧200 vs <200 IU/l) and age (≤35 vs >35 years of age) showed borderline significance with a higher chance of response among all patients (P = 0.056 and P = 0.082, respectively). Next, we investigated the significance of response to IFN therapy by multivariate logistic regression analysis. Both HBeAg and age independently and significantly influenced the outcome of IFN therapy (Table 4).

Discussion

Although IFN is reported to have beneficial effects in the treatment of chronic hepatitis B, the response rate is not high. A metaanalysis published in 1993 reviewed 15 randomized controlled studies involving 837 adult patients who received IFN-α for 4–6 months; loss of HBeAg occurred in 33% of the treated patients.[3] In our study, the response rate to IFN among the HBeAg-positive patients was lower than that in the above studies. The reasons for the difference between studies may

Table 4. Factors associated with response to interferon therapy

Variable	Multivariate odds ratio	95% Confidence interval[a]	*P* value
HBeAg (negative vs positive)	11.1	2.7–46.1	0.0009
Age (≤35 vs >35 years)	5.2	1.3–21.0	0.0209

[a] Values are the odds of having a response to interferon

be differences in ethnic groups and/or in HBV genotype. Kao et al.[11] reported that HBV genotype C, compared to genotype B, was associated with a lower response rate to IFN-α therapy among chronic hepatitis B patients with HBeAg. The response rate among our patients with genotype C was low similar to the results of Kao et al.[11] (response rate; 15%). In our study, in the HBeAg-positive patients, young patients, especially those with a high ALT level at baseline, were significantly more likely to respond to IFN. These prognostic factors were similar to those reported in previous studies,[4–6] although the sample size in our study was small. On the other hand, our previous report[17] showed that 16 of 52 (31%) patients who received IFN-α, given twice per week for 52 weeks, were responders. Therefore, a long-term therapeutic regimen may be necessary to secure a better response.

On the other hand, in the present study the response rate in patients negative for HBeAg was higher than that in those with HBeAg. Previous reports showed that response rates to a 6- to 12-month course of IFN-α in patients with HBeAg-negative CHB ranged from 10% to 47% (average, 24%).[18–21] Moreover, another previous study of ours[22] showed that 9 of 12 (75%) patients who received IFN-β, given twice per week for 24 weeks, responded to the therapy. Considered together, these findings show that 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 patients than in HBeAg–positive patients.[2] The dose of IFN also had little effect, but the duration of therapy (12 vs 5–6 months) was associated with a doubling of sustained response rates.[23]

Our study included two patients with normal ALT levels and 9 patients with undetectable leels of HBV DNA at the commencement of IFN therapy. In these patients, these parameters had decreased by chance at the commencement of IFN therapy, although they had been increased in the 3 months before the treatment. However, the two patients with normal ALT levels at commencement were non-responders. While the response to IFN of patients with normal ALT levels may be poor, that of patients with undetectable levels of HBV DNA at commencement seems similar to other patients. Furthermore, among patients with undatectable levels of HBV DNA at commencement there was no difference in the response rate of those with and without HBeAg.

In our study, no difference in the response to IFN monotherapy was noted between genotypes B and C in patients without HBeAg. Previous reports showed that HBV genotype B was associated with a higher rate of antiviral response to IFN-α treatment in Chinese patients with HBeAg-positive chronic hepatitis B than genotype C.[11,24] It is not clear at present whether this phenomenon applies in patients who are negative for HBeAg.

Recently, Sugauchi et al.[12] proposed that genotype B could be provisionally classified into Ba and Bj subgroups. In Japan, Bj is the major group (93% with genotype B) and most patients with Bj are HBeAg-negative (92%).[25] In our study, six of the seven patients with genotype Bj were HBeAg-negative. Therefore, it is difficult to investigate whether HBV genotype B is associated with a higher rate of antiviral response to IFN treatment than genotype C in Japanese patients with HBeAg-positive chronic hepatitis B.

In our study, HBeAg negativity and younger age were identified as independent and significant determinants of the outcome of IFN therapy. However, an important issue in the treatment of HBeAg-negative chronic hepatitis B is the sustainability of the response to treatment. Our results, which showed a decrease in the response to treatment at long-term follow-up, were similar to those reported by Papatheodoridis et al.[26] In their long-term follow-up of treated patients, it was reported that the sustained response rates decreased from 41% after 6 months to 22% at 2–5 years and thereafter.[26] Lampertico et al.[27] recently reported that 24-month IFN treatment resulted in sustained disease suppression in a significant proportion of patients with HBeAg-negative chronic hepatitis B.[27] Long-term IFN therapy may improve the response to IFN therapy in HBeAg-negative patients. On the other hand, lamivudine, another approved treatment for chronic hepatitis B, requires long-term continuous therapy and could potentially be associated with the development of viral resistance.[28] Considering these aspects of treatment modalities, patients of younger age may require long-term IFN therapy.

In conclusion, we investigated the efficacy of 6-month IFN therapy for Japanese patients. The response rate to

IFN among HBeAg-positive patients was low. In this group, patients of young age with high ALT levels were significantly more likely to respond to IFN monotherapy than other patients. On the other hand, the response rate to IFN among HBeAg-negative patients was high. Multivariate analysis identified HBeAg negativity and young age as independent determinants of the outcome of 6-month IFN therapy. Further studies, such as longer-term therapy (over 1 year) may be necessary in order to confirm these findings and establish the true response to IFN therapy.

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Efficacy of interferon retreatment after relapse for chronic hepatitis C patients with biochemical response after first interferon therapy

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Background. With respect to interferon (IFN) treatment for chronic hepatitis C, normalization of alanine aminotransferase (ALT) as well as clearance of hepatitis C virus (HCV)-RNA after IFN therapy is important. It has been shown that the incidence of hepatocellular carcinoma (HCC) in patients with normal ALT is significantly lower than that in those with elevated ALT after IFN therapy. We assessed the efficacy of IFN retreatment for chronic hepatitis C patients who had a biochemical response (BR) after the first IFN therapy and reelevated ALT during follow up, by a case control study. ***Methods.*** Fifteen patients (case group; group 1) enrolled in this study showed a BR after the first IFN therapy and reelevated ALT during follow up. Next, we retrospectively selected 30 patients (control group; group 2) with no response (NR) after the first IFN therapy. Group 2 patients were matched 1:2 with group 1 patients for sex and age. All patients were given intramuscular injections of human lymphoblastoid IFN alpha daily (6 MU) for 8 weeks and then three times a week for 16 weeks. We compared the clinical and biological differences between group 1 patients and group 2 patients. Virological response (VR) was defined when serum HCV-RNA showed negativity more than 6 months after the completion of IFN therapy. BR was defined when ALT values remained normal during more than 6 months in spite of positive serum HCV-RNA, by reverse-transcription nested polymerase chain reaction, 3 and 6 months after the completion of IFN therapy. NR was defined as any response except for VR or BR. ***Results.*** The rate of patients showing VR in this study was 6.7% (1/15) in group 1 and 13.3% (4/30) in group 2. There was no significant difference between the groups with respect to VR. BR occurred in 73.3% (11/15) of patients in group 1, but in only 3.3% (1/30) of patients in group 2 ($P = 0.0002$). ***Conclusions.*** We conclude that IFN retreatment is one of the effective strategies with which to achieve BR again in HCV-positive patients who had a BR after their first IFN therapy and reelevation of ALT during follow up.

Key words: chronic hepatitis C, interferon retreatment, biochemical response

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Introduction

Currently, as shown in many studies, interferon (IFN) is the only drug that induces viral clearance and marked biochemical and histological improvement in patients with chronic hepatitis C.[1–12] In these many studies, hepatitis C virus (HCV)-RNA clearance rates were reported to be about 30%–40% in patients treated with a course of IFN of less than 6 months. However, with respect to IFN treatment for chronic hepatitis C, normalization of alanine aminotransferase (ALT) as well as clearance of HCV-RNA after IFN therapy is important. Previous studies have indicated that the incidence of hepatocellular carcinoma (HCC) was lower in patients with normal ALT levels than in patients with abnormal ALT levels.[13–17] Thus, apart from eradication of the virus, patients with normalization of ALT levels have a lower possibility of HCC appearance than patients showing no response (NR) after IFN therapy. In fact, it has been shown that the HCC rate in patients with a biological response (BR) was significantly lower than that in those with NR after IFN therapy. Moreover, Mathurin et al.[18] reported that HCV-positive patients with normal serum ALT showed weaker histological activity and lower fibrosis scores, and the progression rate of fibrosis was twice as slow as that in HCV patients with elevated ALT. Therefore, attainment of ALT normalization

after IFN therapy appears to be valuable in chronic hepatitis C patients who remain viremic. In fact, about 10%–20% of chronic hepatitis patients treated with IFN had a BR.[19] However, in patients with a BR after IFN therapy, the serum ALT level was often reelevated during the follow-up period. When such patients are retreated, it is problematic as to whether IFN therapy should be used again. We assessed the efficacy of IFN retreatment for chronic hepatitis C patients who showed a BR after the first IFN therapy and reelevated ALT during follow up.

Subjects, materials, and methods

Patient populations

Fifteen patients (case group; group 1) were enrolled in this trial from 1994 to 1999. Enrollment criteria for inclusion in the case group were the following: (1) efficacy of first IFN therapy showed BR. (2) Average serum ALT showed values more than the upper normal limits (normal range of ALT, 12–50IU/l) for more than 3 months before inclusion in this trial. (3) Liver biopsy taken within 3 months prior to this trial showed histological features of chronic active hepatitis. (4) No treatment with corticosteroids, immunosuppressive agents, or antiviral agents within 12 months prior to this trial. (5) No hepatitis B surface antigen (HBsAg), as determined by radioimmunoassay. (6) No antinuclear antibody (ANA) or antimitochondrial antibody (AMA) in the serum, as determined by immunofluorescence on rat liver and kidney. The following patients were excluded from the study: (1) those with liver cancer or severe liver failure; (2) pregnant women; (3) febrile patients, patients with leukocyte counts less than 3000/μl, and those with platelet counts less than 7000/μl; (4) patients with renal disorders; and (5) patients with a past history of hypersensitivity reactions to biological preparations such as vaccines.

To compare the clinical and biological differences between patients (group 1) with a BR after the first IFN therapy and those with NR after the first IFN therapy, we retrospectively selected 30 patients, matched 1:2 with group 1 patients for sex and age (control group; group 2). Patients in group 2 were selected from among the 106 patients who satisfied the enrollment criteria of group 1 and were retreated in the same way as group 1 patients, with human lymphoblastoid IFN alpha.

Study protocol

IFN for retreatment was prepared by Sumitomo Pharmaceutical (Tokyo Japan). IFN vials contained 6 MU of human lymphoblastoid IFN alpha. The patients were given intramuscular injections of 6 MU daily for 8 weeks and then three times a week for 16 weeks. Our study was approved by the institutional review board of our hospital. The physicians in charge explained the purpose and method of this clinical trial, as well as potential adverse reactions, to each patient, who gave their informed consent for participation. Virological response (VR) was defined as HCV-RNA negativity, determined by reverse-transcription nested polymerase chain reaction (RT-nested PCR),[20] more than 6 months after the completion of IFN therapy. Biochemical response (BR) was defined when ALT values remained normal during more than 6 months in spite of positive serum HCV-RNA, determined 3 and 6 months after the completion of IFN therapy. Patients who did not fulfill these VR and BR criteria were classified as showing no response (NR).

Blood testing

Blood samples were obtained just before therapy and were stored at −80°C. By use of these blood samples, HCV-RNA levels before IFN therapy were analyzed by a branched DNA probe assay (b DNA probe assay, version 2.0; Chiron; Dai-ichi Kagaku, Tokyo, Japan) and the results were expressed as mega equivalents per milliliter (Meq/ml).[21] Serum ALT concentrations were measured at least once per month for 6 months prior to the initiation of IFN therapy, one to two times per month while IFN was being administered, and once per month thereafter. Blood samples of patients that showed a serum HCV-RNA level of less than 0.5Meq/ml during and after IFN therapy were measured again by the RT-nested PCR. Serum HCV-RNA levels were measured at least once per 2 or 3 months during and after completion of IFN therapy. HCV genotype was examined by a PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously.[22]

Histopathological examination of liver biopsy specimen

Liver biopsy specimens were obtained percutaneously or at laparoscopy, using a modified Vim Silverman needle with an internal diameter of 2mm (Tohoku University style; Kakinuma, Tokyo, Japan). Baseline liver histology of chronic hepatitis prior to IFN therapy was classified, according to the extent of fibrosis, into three stages: mild, periportal expansion; moderate, portoportal septa: and severe, portocentral linkage or bridging fibrosis.[23]

Statistical analysis

Baseline characteristics and treatment differences between groups were analyzed using Fisher's exact test

(two-tailed) or the Wilcoxon rank sum test, as appropriate. Finally, we used logistic regression analysis to determine those factors that contributed to a BR after the IFN retreatment. A P value of less than 0.05 was chosen to indicate statistical significance. The SPSS software package (SPSS, Chicago, IL, USA) was used to perform the analyses.

Results

Clinical background

The background characteristics and baseline measurements for each group are summarized in Table 1. There were no significant differences between the two groups in sex, age, serum AST levels, serum ALT levels, serum HCV-RNA levels, HCV genotype, the total dose of first IFN administration, or the histopathological diagnosis of biopsied liver specimens prior to IFN retreatment.

Safety profile and efficacy of IFN therapy

Fifteen patients in group 1 and 30 patients in group 2 (selected retrospectively by matching 1:2 with group 1 patients for sex and age) were studied. None of the patients in group 1 or group 2 was withdrawn from this study. Table 2 shows the effect of IFN retreatment according to the effect of the first IFN therapy. The rate of patients showing VR in this study was 6.7% (1/15) in group 1 and 13.3% (4/30) in group 2. There was no significant difference with respect to VR between the groups. BR occurred in 73.3% (11/15) of patients in group 1, but in only 3.3% (1/30) of patients in group 2. The BR rate of patients in group 1 was significantly higher than that of patients in group 2 ($P = 0.0002$).

We assessed the univariate analysis of clinical factors that contributed to the BR after IFN retreatment, excluding the five patients who showed VR after IFN retreatment (Table 3). Among the tested parameters, attainment of BR after the first IFN therapy had a significant effect on the attainment of BR after IFN retreatment in this trial. Age, sex, liver histology, ALT, and genotype had no significant effect on the attainment of BR after IFN retreatment in this trial.

Period of biochemical response after IFN therapy

In group 1 patients, the median period of BR after the first IFN therapy was 24 months (range, 7–50 months). Eight of 11 patients in group 1 with BR after IFN retreatment had reelevated ALT after prolonged follow up, and the remaining 3 patients showed continued normalization of ALT. The median period of BR after IFN retreatment in these 11 patients in group 1 was 25 months (range, 7–52 months).

Discussion

About 30%–40% of chronic hepatitis C patients treated with IFN monotherapy show eradication of serum HCV-RNA after the end of the IFN therapy.[1–12] Espe-

Table 1. Comparison of clinical, virological, and histological pretreatment features of the patients

Effect of first IFN therapy	Group 1; BR (n = 15)	Group 2; NR (n = 30)	P value
Age (years)[a]	41–68 (52)	25–63 (52)	Matched
Sex (male/female)	13/2	26/4	Matched
Total dose of first IFN (MU)[a] (<500/≧500)	168–938 (528) 6/9	168–1040 (480) 17/13	0.353
Period between first and second IFN (months)[a] (<24/≧24)	18–72 (39) 3/12	12–88 (32) 9/21	0.722
Histology (staging; slight/moderate or severe)	10/5	17/13	0.748
Genotype (1b/2a or 2b)	13/2	22/8	0.456
HCV-RNA (Meq/ml)[a] (<5/≧5)	<0.2–30 (11.0) 5/10	<0.2–29.0 (4.0) 16/14	0.342
AST (IU/l)[a]	20–96 (54)	18–401 (74)	0.716
ALT (IU/l)[a]	9–214 (94)	19–458 (111)	0.518

ALT, alanine aminotransferase (normal range, 6–50 IU/l); AST, aspartate aminotransferase (normal range, 11–38 IU/l); BR, biochemical response; NR, no response; IFN, interferon
[a] Data values are expressed as ranges (medians)

cially, in patients with genotype 1b and a high load of HCV-RNA, the clearance rate of HCV-RNA is less than 10% with the usual 6-month course of IFN monotherapy. In these IFN-resistant patients, the eradication rate of serum HCV-RNA is at most 20%–50% by the latest prolonged IFN therapy,[24–27] combination therapy with IFN/ribavirin,[28–31] or pegylated IFN administration.[32,33]

However, apart from eradication of the virus, patients with normalization of ALT levels have a lower possibility of HCC appearance than patients showing elevated ALT after IFN therapy. That is, attainment of ALT normalization after IFN therapy appears to be valuable in chronic hepatitis C patients who remain viremic. In patients with a BR after IFN therapy, the serum ALT level was often reelevated during the follow-up period. We have reported that the incidence of ALT reelevation was about 10% per year in the patients treated with IFN who showed a BR.[19] If patients with ALT reelevation after an IFN-induced BR could attain BRs at a high rate again after IFN retreatment, it might be feasible to treat them with IFN again.

In the present study, we assessed the factors related to BR after IFN retreatment. This case-control study suggests that patients with a BR induced by the first IFN therapy show BRs again, at a high rate, after a second IFN treatment. We selected the 30 subjects in the control group from 106 subjects who satisfied the study criteria. Therefore, the total number of subjects consisted of 15 with BR and 106 with NR after the first IFN therapy. In this total of 121 subjects, we assessed factors related to BR after IFN retreatment by multivariate analysis. According to this analysis, the risk ratio for BR appearance after IFN retreatment in patients with a BR after the first IFN therapy was 13.5 compared with patients with NR after the first IFN therapy. This means that repeated IFN would maintain ALT normalization at a high rate, in spite of positive serum HCV-RNA, in patients with a BR by the first IFN therapy.

At present, combined IFN and ribavirin therapy is a standard therapy for chronic hepatitis C patients with genotype 1b and a high load of HCV-RNA. There is an interesting problem in how the results of this present study are to be interpreted when ribavirin therapy is available. Therefore, we retrospectively assessed the efficacy of combination therapy for 14 patients with BR and 47 patients with NR after a first IFN monotherapy

Table 2. Effect of interferon retreatment according to effect of first interferon therapy

Effect of IFN retreatment	Effect of first IFN therapy	
	Group 1; BR (n = 15)	Group 2; NR (n = 30)
VR[a]	1 (6.7%)	4 (13.3%)
BR[a]	11 (73.3%)	1 (3.3%)
NR[a]	3 (20%)	25 (83.3%)

[a] VR, virological response; BR, biochemical response; NR, no response

Table 3. Analysis for the predictors of BR after IFN retreatment

Factor	BR	NR	P value
No. of patients	12	28	
Age (years)[†]	41–61 (48)	25–67 (53)	
<50	7	14	
≧50	5	14	0.492
Sex (male/female)	12/0	24/4	0.804
Histology (staging; slight/moderate or severe)	9/3	16/12	0.291
Genotype (1b/2a or 2b)	11/1	21/7	0.135
HCV-RNA (Meq/ml)[a]	<0.2–30 (6.0)	<0.2–29.0 (5.8)	
<5/≧5	5/7	12/16	0.944
ALT (IU/l)[a]	18–262 (88)	24–458 (114)	
<100/≧100	7/5	12/16	0.372
HCV-RNA[b]			
−/+	8/4	19/9	0.619
ALT (IU/l)[b]			
Normal/Abnormal	12/0	21/7	0.818
Efficacy of first IFN			
BR/NR	11/1	3/25	0.0002

[a] Values at the starting point of IFN retreatment
[b] Values at the end of IFN retreatment
ALT, alanine aminotransferase; BR, biochemical response; IFN, interferon; NR, no response

in our hospital. The rate of BR after combination therapy was 50% (7/14) in patients with a BR after the first IFN therapy and 15% (7/47) in patients with NR; the rate of BR after the combination therapy in patients with a BR after the first IFN monotherapy was significantly higher, by Fishers' exact test ($P = 0.029$). This means that combination therapy, as did IFN monotherapy, would maintain ALT normalization at a high rate in patients with a BR by the first IFN therapy.

The mechanism of the induction of BR remains obscure. However, previous studies have identified various factors that could predict a BR after IFN therapy. The factors that predicted BR were as follows: prolonged IFN therapy,[10] normalization of ALT during IFN therapy,[19] slight fibrosis of the liver before IFN therapy,[19] and disappearance of the major clone in hypervariable region 1 after IFN therapy.[34] Kasahara et al.[10] also reported that prolonged IFN therapy would assist in the achievement of BR. Nishiguchi et al.[34] reported changes in hypervariable region 1 in patients with chronic hepatitis C of genotype 1b showing a BR to IFN.

In conclusion, IFN retreatment is one of the effective strategies with which to achieve a BR in patients who showed a BR after the first IFN therapy and had reelevated ALT during follow up.

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Journal of Gastroenterology

Efficacy of lamivudine treatment in Japanese patients with hepatitis B virus-related cirrhosis

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Background. Several clinical trials have suggested that lamivudine therapy is effective in patients with hepatitis B virus (HBV)-related cirrhosis. However, there are few studies of lamivudine therapy in Japanese patients with HBV cirrhosis. The aim of this study was to evaluate the efficacy of lamivudine therapy in Japanese patients with cirrhosis, and to evaluate the clinical course after the emergence of YMDD mutants. ***Methods.*** Fifty-four consecutive adult Japanese patients with HBV-related cirrhosis were enrolled and continuously treated with lamivudine, daily for 6–35 months (median, 25 months). Twelve of the 54 patients were hepatitis B envelope antigen (HBeAg)-positive. The clinical courses of 21 of the patients were evaluated using the Child-Pugh-Turcott (CPT) score. ***Results.*** Lamivudine suppressed serum HBV-DNA to undetectable levels (<3.7 LGE/ml) in 77.8% of patients at 12 months and in 61.3% at 24 months. Before the emergence of YMDD mutants, clinical improvement, defined as a decrease in the CPT score of 2 points or more, was apparent in 6 of 21 (29%) patients. No change in CPT score was evident in 14 of 21 patients (67%). YMDD mutants emerged in 19 of 54 (35%) patients. The cumulative emergence rates increased each year. The emergence rate of YMDD mutants in patients with HBV cirrhosis was higher than that in patients with chronic hepatitis. After the emergence of YMDD mutants, 3 of 12 (25%) patients with YMDD mutants showed CPT score increases of 2 points or more. ***Conclusions.*** Lamivudine therapy improved the clinical course in some cirrhotic patients. However, in patients with Child's B and C cirrhosis, the emergence of YMDD mutants sometimes led to deterioration of liver function.

Key words: HBV, cirrhosis, lamivudine, Child-Pugh-Turcott score

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Introduction

Lamivudine, an oral cytosine nucleoside analogue, potently inhibits hepatitis B virus (HBV) replication by interfering with HBV reverse transcriptase activity.[1–4] Several studies have reported the effectiveness of lamivudine in the suppression of HBV replication, improvement of transaminase levels and liver histology, and enhancement of the rate of loss of hepatitis B envelope antigen (HBeAg).[3–7]

Recently, several studies have suggested the effectiveness of lamivudine therapy for patients with HBV-related cirrhosis, especially those with decompensated cirrhosis.[8–15] Lamivudine therapy for cirrhotic patients may also be recognized as a bridge to more definitive therapy, such as liver transplantation. However, in several countries, including Japan, liver transplantation is not available because of the insufficiency of donors, and even in other countries, many patients have to wait long periods for liver transplantation. Therefore, lamivudine has been used for patients with HBV-related cirrhosis for long durations. Although several studies showed the efficacy of lamivudine therapy for patients with HBV cirrhosis in the United States and European countries,[9–15] there are few studies of lamivudine therapy in which all patients were Japanese, with HBV of genotype C, with liver cirrhosis. In this regard, a major problem with the long-term use of lamivudine is the development of viral resistance, associated with increases in HBV-DNA and serum transaminase levels.[16–18] There are few studies that have addressed this issue in Japanese patients with cirrhosis.

The aims of the present study were: (1) to assess the benefits of long-term lamivudine therapy for Japanese patients with HBV-related cirrhosis, (2) to evaluate the progress after the appearance of YMDD mutants, and (3) to determine differences in the emergence rate of YMDD mutants between patients with chronic hepatitis and those with liver cirrhosis.

Table 1. Patient characteristics at the commencement of lamivudine therapy

	LC (n = 54)	CH (n = 54)	P value
Sex (male/female)	45/9	45/9	Matched
HBeAg-positive (number; %)	12 (22.2)	12 (22.2)	Matched
Age (years)[a]	47 (28–63)	46 (27–63)	Matched
Genotype (A/B/C/D)	1/3/48/0	1/6/46/1	NS
HBV-DNA (LGE/ml)	6.7 (3.7–8.7)	7.0 (3.7–8.7)	NS
T-Bilirubin (mg/dl)[a]	0.8 (0.3–7.8)	0.7 (0.2–10.6)	NS
AST (IU/l)[a]	54 (25–419)	73.5 (24–1493)	NS
ALT (IU/l)[a]	73 (16–795)	105 (17–2142)	0.031
Albumin (g/dl)[a]	3.8 (2.4–4.8)	4.0 (3.0–4.5)	NS
Child classification (A/B/C)	38/11/5	—	—
Duration (month)[a]	25 (6–35)	31 (13–83)	0.006

NS, not significant
[a]Data values are medians (ranges)

Patients and methods

Patients

Between February 1998 and April 2002, 54 consecutive adult Japanese patients with HBV-related liver cirrhosis were enrolled in this study at Toranomon hospital, Tokyo. All patients fulfilled the following criteria: (1) hepatitis B surface antigen (HBsAg) present in serum; (2) HBV-DNA positivity by quantitative assay; (3) presence of liver cirrhosis confirmed by laparoscopy, liver biopsy, and/or ultrasonography; (4) absence of hepatoma; (5) absence of co-infection with HCV or HIV; and (6) absence of a past history of treatment with any nucleoside analogue.

The baseline characteristics of the 54 patients included in the study are shown in Table 1. All patients were Japanese; 45 were men, and 9, women. Twelve patients were HBeAg-positive and 42 were HBeAg-negative. Forty-eight patients had genotype C, 3 had genotype B, 1 had genotype A, and in 2, the genotype was unknown.

To compare the emergence rate of YMDD mutants between patients with HBV-related chronic hepatitis and those with liver cirrhosis, 54 patients were selected from among the 217 patients with chronic hepatitis B on lamivudine therapy in our hospital, because they matched patients with HBV-related liver cirrhosis with respect to sex, age, and HBeAg status.

Methods

All patients were treated with lamivudine 100 mg or 150 mg orally, given daily continuously for at least 6 months, after providing their informed consent.

Clinical and laboratory assessments were performed once a month. Clinical improvement was defined as a decrease in the Child-Pugh-Turcott (CPT) score[19] of at least 2 points; no change was defined as an increase or decrease of 1 point or no change in score; and worsening was defined as an increase of at least 2 points. Adverse effects were monitored clinically by careful interview and medical examination once a month. Abdominal ultrasonography was performed every 6 months to assess for development of hepatoma.

After the emergence of a YMDD mutant (see "Results"), we provided interferon (IFN) therapy for those patients who fulfilled the following criteria: (1) aspartate transaminase (AST) and alanine transaminase (ALT) levels of 100 IU/l or more, or total bilirubin (T-Bil) level of 1.3 mg/dl or more; (2) platelet counts of 50 × 10^3/μl or more; and (3) neutrophil counts of 1000/μl or more. The majority of such patients received IFN-α, which was administered for 4 weeks, at 3 million units (MU) one daily, and for 20 weeks at 3 MU three times weekly. During therapy, the dose of IFN was reduced or the treatment was withdrawn altogether when the platelet count or neutrophil count decreased below the criteria.

Laboratory and virological testing

Routine biochemical test were performed at least once a month before and during therapy, using standard procedures, and the CPT score was determined. Serial blood samples were taken before and during therapy every month and stored at −80°C until used for HBV mutant analysis. HBeAg and antibody to HBeAg (anti-HBe) were determined by radioimmunoassay kits (Abbott Diagnostics, Chicago, IL, USA). HBV-DNA was measured by a transcription-mediated amplification and hybridization protect assay (TMA-HPA; Chugai Diagnostics Science, Tokyo, Japan).[20] Mutations in the YMDD motif in the polymerase gene were determined using the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), by a method described previously.[17] Lamivudine resistance

was determined annually before the development of mutations, and, if mutation appeared, the time of appearance of resistance was confirmed by monthly measurement.

Data analysis

Kaplan-Meier analysis and the log-rank test were applied to estimate and compare the rate of viral resistance between HBeAg-positive and -negative patients. A two-tailed P value of less than 0.05 was considered statistically significant. Non-parametric tests, including the χ^2 test, Fisher's exact probability test, and the Mann-Whitney U-test were used to compare the background characteristics and efficacy.

Results

Serum HBV-DNA and ALT concentrations

Figure 1 shows the HBV-DNA loss rates. Lamivudine suppressed serum HBV-DNA to undetectable levels (<3.7 LGE/ml) in 77.8% of the patients at 6 months, 77.8% at 12 months, 60.5% at 18 months, 61.3% at 24 months, and 47.8% at 30 months. In all patients, HBV-DNA levels decreased significantly within 4 weeks of therapy. However, HBV-DNA loss rates tended to decrease each year. Figure 1 also shows the ALT and AST normalization rates, and shows that the significant improvements were sustained during the follow-up period. After the emergence of a YMDD mutant, 12 patients received IFN therapy. When we evaluated the efficacy of lamivudine treatment for patients without IFN therapy, lamivudine suppressed HBV-DNA to undetectable levels in 77.8% of the patients at 6 months, 98% at 12 months, 91% at 18 months, 75% at 24 months, and 72% at 30 months. The ALT normalization rate was 89% of the patients at 6 months, 80% at 12 months, 86% at 18 months, 86% at 24 months, and 78% at 30 months.

HBeAg seroconversion

Of the 12 patients who were positive for HBeAg, seroconversion occurred in 6 during the 35-month treatment period. Seroconversion in the 6 patients was identified at 18, 19, 21, 21, 23, and 29 months, respectively. Among the 6 patients, 2 had HBeAg reappearance after emergence of virological breakthrough.

Lamivudine resistance

YMDD mutants were not detected in any of the pretreatment serum samples. Nineteen (35%) patients developed mutations in the YMDD motif. The characteristics of the 19 patients with YMDD mutants are shown in Table 2. The cumulative emergence rates were 16% at 1 year, 39% at 2 years, and 47% at 2.5 years (Fig. 2). Lamivudine resistance was confirmed by demonstration of the YIDD ($n = 7$), YVDD ($n = 2$), or YIDD and YVDD simultaneously ($n = 8$) form of HBV mutant when mutations in the YMDD motif initially appeared.

Univariate analysis showed that HBeAg was the only predictive factor for the appearance of YMDD mutants (Table 2, Fig. 3). The differences were not statistically

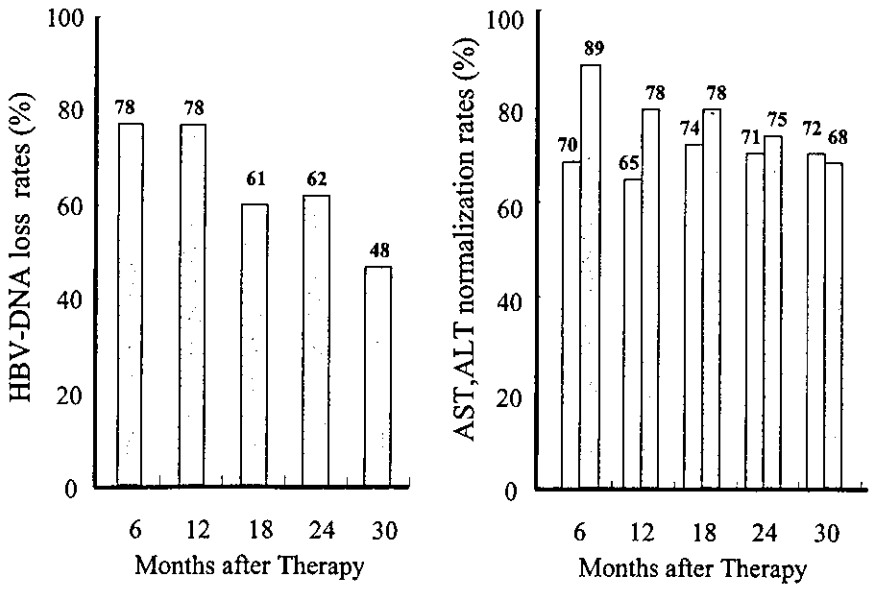

Fig. 1. A Hepatitis B virus (*HBV*)-DNA loss rates. **B** Alanine transaminase (*ALT*; *gray bars*) and aspartate transaminase (*AST*; *white bars*) normalization rates. The *numbers above each bar* represent the actual rate

Table 2. Comparison of patients with and without YMDD mutant during lamivudine therapy

	YMDD mutant (n = 19)	No YMDD mutant (n = 35)	P value
Sex (male/female)	17/2	28/7	NS
HBeAg-positive (number; %)	8 (42.1)	4 (11.4)	0.030
Age (years)[a]	47 (28–61)	48 (31–63)	NS
Genotype (A/B/C/D)	1/2/16/0	0/1/31/1[b]	NS
HBV-DNA (LGE/ml)[a]	6.6 (3.9–8.9)	6.7 (3.7–8.4)	NS
AST (IU/l)[a]	47 (25–131)	67 (31–419)	NS
ALT (IU/l)[a]	61 (24–201)	87 (16–795)	NS
Albumin (g/dl)[a]	3.8 (2.8–4.3)	3.9 (2.4–4.8)	NS
Time to emergence of YMDD mutant (month)[a]	14 (4–30)	—	—

[a] Data values are medians (ranges)
[b] Genotype was unknown in two patients

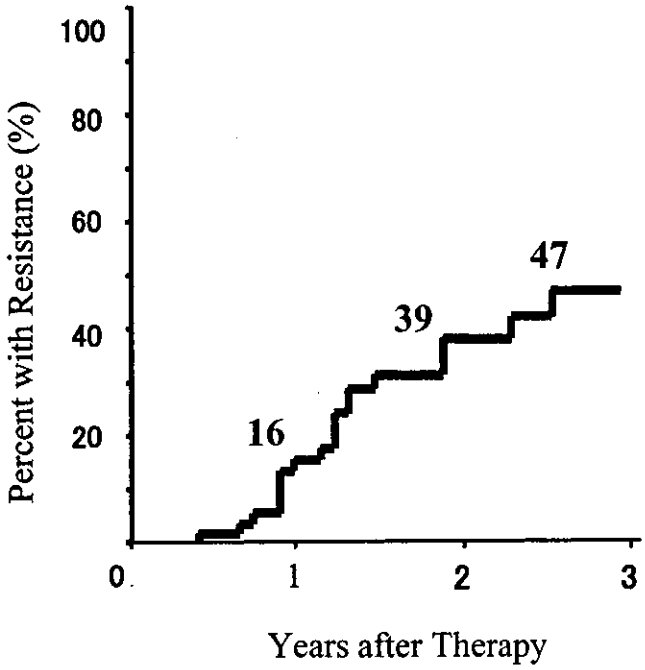

Fig. 2. Cumulative percentages of 54 patients who showed viral resistance during treatment with lamivudine (Kaplan-Meier analysis). *Numbers* represent the actual percentages for the indicated intervals

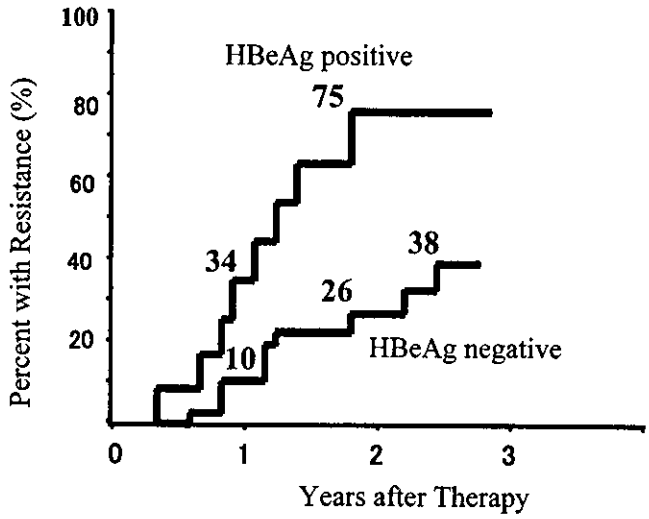

Fig. 3. Cumulative percentages of hepatitis B envelope antigen (*HBeAg*)-positive and -negative patients who showed viral resistance during treatment with lamivudine (Kaplan-Meier analysis). *Numbers* represent the actual percentages for the indicated intervals

significant for age, sex, pre-treatment HBV-DNA level, or pre-treatment ALT and AST levels. The cumulative emergence rates in patients with HBeAg were 34% at 1 year, 75% at 2 years, and 75% at 2.5 years. In contrast, in patients without HBeAg, the rates were 10% at 1 year, 26% at 2 years, at 38% at 2.5% years.

The rate of appearance of YMDD mutants during treatment was compared between patients with liver cirrhosis and those with chronic hepatitis (Table 1, Fig. 4). There was a difference in the frequency of lamivudine resistance (P = 0.03). By 1 year, 16% of cirrhotic patients, but only 10% of chronic hepatitis patients, had developed viral resistance. The proportion of cirrhotic patients with YMDD mutants rose to 39% at 2 years and 47% at 2.5 years. In contrast, only 23% of the patients with chronic hepatitis developed resistance after 2.5 years. In patients with chronic hepatitis, 18 had F0–1 (33.3%), 20 had F2 (37%), 10 had F3 (18.5%), and the stage in the remaining patients was unknown. In more than 70% of patients with chronic hepatitis, hepatic fibrosis was mild (F0–2). Thus, it was considered that our study compared only cases classified as F4 with those classified as F0–2.

Appearance of breakthrough hepatitis

In 18 (95%) of the 19 patients with YMDD mutants, breakthrough hepatitis appeared after emergence of the

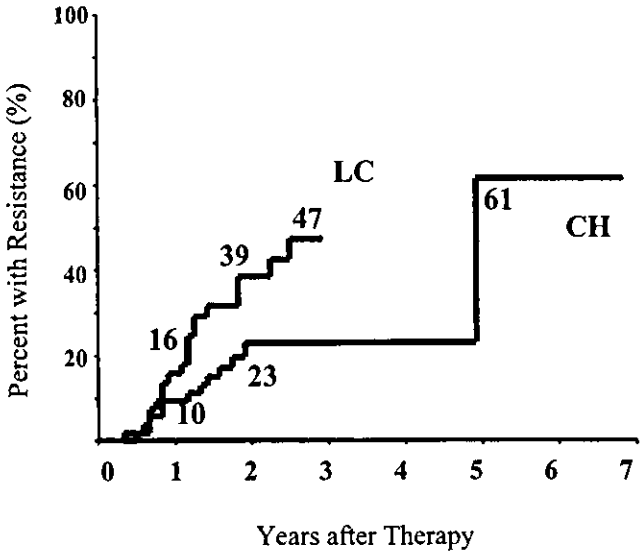

Fig. 4. Cumulative percentages of patients with liver cirrhosis (*LC*) and those with chronic hepatitis (*CH*) who showed viral resistance during treatment with lamivudine (Kaplan-Meier analysis). *Numbers* represent the actual percentages for the indicated intervals

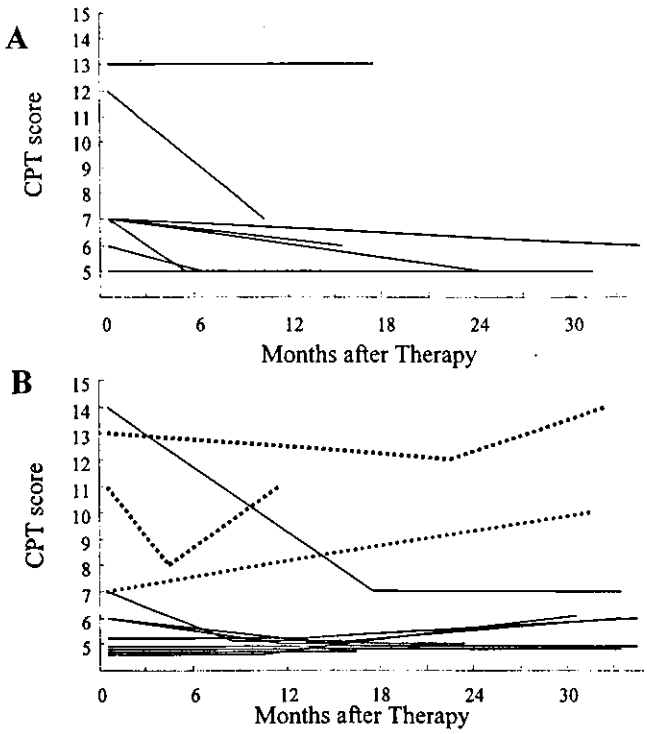

Fig. 5A,B. Individual serial Child-Pugh-Turcott (*CPT*) scores for patients treated with lamivudine. **A** Patients without breakthrough hepatitis (n = 9), and **B** patients with breakthrough hepatitis (n = 12). Clinical response was defined as a decrease in the CPT score of at least 2 points. Where overlap at a particular numerical score occurred, the lines have been slightly separated in order to allow better discrimination of the distinct responses. the *broken lines* indicate patients with severe acute exacerbation and jaundice after emergence of YMDD mutants

YMDD mutant. Only 1 patient did not show an increase in AST and ALT activity during lamivudine therapy after emergence of the YMDD mutant. Of the 18 patients with breakthrough hepatitis, 8 were HBeAg-positive and 10 were HBeAg-negative. Twelve of the 18 patients received IFN therapy. Three of these patients, who were all HBeAg-positive, had severe acute exacerbation and jaundice. Breakthrough hepatitis in these patients was controlled by IFN therapy.

Changes in Child-Pugh-Turcott score

Unfortunately, pre-treatment prothrombin times were not available in about half of the patients; therefore, the CPT score was analyzed in only 21 of the 54 patients during the lamivudine therapy. Nine of the 21 were Child's A (CPT score, 5–6), 7 were Child's B (CPT score, 7–9), and 5 were Child's C (CPT score, 10–15). In the 33 patients who could not be tested for CPT, 29 were Child's A, 4 were Child's B, and none was Child's C. In the 21-patient group, the percentage of patients with Child's C was significantly higher than that in the 33-patient group. There were no significant differences between the two groups for age, sex, pre-treatment HBV-DNA level, or pre-treatment ALT, AST, and albumin levels. Twenty-one patients were analyzed for improvement in liver function accompanying the decrease in CPT score. Before the emergence of the YMDD mutants, clinical improvement, defined as a decrease in the CPT score of at least 2 points, was evident in 6 of 21 (29%) patients. Three of the 6 had Child's C and the other 3 had Child's B cirrhosis at baseline. In 14 of the 21 (67%) patients, the CPT score decreased by only 1 or 0 points. Only 1 patient showed deteriorated condition, due to the appearance of hepatocellular carcinoma (HCC), which required surgical treatment.

Twelve of the 21 patients developed YMDD mutants and breakthrough hepatitis. Figure 5 shows the individual CPT scores over time for patients with and without YMDD mutants. In 3 of the 12 (25%) patients with YMDD mutants, CPT scores increased by 2 points or more. Two of these three patients had severe acute exacerbation and jaundice and received IFN therapy, as described above. These 2 patients were Child's C at baseline.

Appearance of HCC

During the follow-up period, HCC was detected in 2 of the 54 (3.7%) patients. One was a 53-year-old man (genotype C and HBeAg-negative). His CPT score

was 13. HCC appeared after 19 months of lamivudine therapy. The other patient was also a 53-year-old man (genotype C and HBeAg-positive). His CPT score was 7. HCC appeared after 14 months of therapy. He had breakthrough hepatitis, and the CPT score increased by 2 points after the breakthrough hepatitis.

Survival rates

All patients remained alive during the follow-up period. No patient underwent liver transplantation. There were no significant side effects associated with prolonged lamivudine therapy.

Discussion

The benefit of lamivudine in patients with compensated HBV-related liver disease has been suggested by several multicenter studies.[2-6] The use of lamivudine in severely decompensated HBV cirrhosis was also recently examined in several published studies, which suggested lamivudine could be safely given and was effective.[8-16] However, there are few studies of lamivudine therapy in Japanese patients with cirrhosis. Our study demonstrated that lamivudine therapy for Japanese patients with HBV-related liver cirrhosis was well tolerated and led to a significant reduction in levels of transaminases and HBV-DNA in patients with HBV cirrhosis, prior to the emergence of YMDD mutants.

However, HBV-DNA loss rates tended to decrease year by year; for example, the rates were 60.5% at 18 months and 47.8% at 30 months, because YMDD mutants developed in some patients when lamivudine was administered for more than 6 months. Compared with HBV-DNA, the high normalization rate of transaminases (about 80%) was sustained during follow-up: after the emergence of YMDD mutants almost all patients preserved their liver function with further treatment.

Of the 54 patients with HBV-related cirrhosis, 19 (35%) developed YMDD mutants after a median of 14 months of lamivudine treatment. The emergence of YMDD mutants is well-described to be associated with high baseline HBV-DNA levels, high ALT levels, and HBeAg-positivity among patients with mainly chronic hepatitis.[18,21-23] In our HBV cirrhosis patients, we identified only HBeAg as an associated factor. High HBV DNA level was not a predictive factor in our study, because the HBV DNA level in cirrhotic patients was lower than that in non-cirrhotic patients. In this study, a high HBV DNA level was detected in 7 of 54 (13%) patients with cirrhosis and 57 of 199 (29%) patients in the non-cirrhotic group. Therefore, HBV DNA level could be excluded as a risk factor. The duration and rate of emergence of YMDD mutants in our study were the same as those previously reported.[6-16] However, we compared 54 patients with cirrhosis with patients with chronic hepatitis who were matched for age, sex, and HBeAg status, with respect to the rate of emergence of YMDD mutants. We found that YMDD mutants appeared more frequently in patients with cirrhosis than in those with chronic hepatitis, especially in the first 3 years. Another study[13] showed no difference in the emergence of YMDD mutants between patients with cirrhosis and those with chronic hepatitis. However, the sample number was smaller than in our study, and the subjects were not matched for clinical factors. The reason for the more frequent emergence of YMDD mutants in cirrhosis than in chronic hepatitis remains obscure, and further studies are necessary to investigate this issue.

We analyzed the CPT score in 21 of 54 patients during lamivudine therapy. Yao and Bass[8] reported 13 patients with decompensated HBV cirrhosis (CPT score >10), who were treated with lamivudine for a mean of 17.5 months. In 9 (69%) of their patients, the CPT score improved, with a decrease of 3 points. Kapoor et al.[14] reported 18 patients with cirrhosis who were treated with lamivudine for a mean of 17.9 months, and the mean CPT score improved from 8.3 to 6.7. Hann et al.[13] reported that CPT scores in 23 (31%) of 75 patients with HBV cirrhosis improved, with a decrease of 2 points. Our result was consistent with those of the above earlier studies, and suggested that lamivudine is effective in Japanese patients with liver cirrhosis.

Twelve of 21 patients whose CPT scores were calculated developed YMDD mutants and breakthrough hepatitis. A few reports have discussed the clinical course and changes of CPT scores after the emergence of YMDD mutants. Villeneuve et al.[9] reported 3 patients who developed breakthrough without significant change in their CPT scores. However, Fontana et al.[15] reported 2 of 22 patients with breakthrough who died due to the breakthrough infection and multisystem organ failure. In our study, after the emergence of the YMDD mutants, CPT scores in patients with Child's A cirrhosis were not significantly changed. On the other hand, in patients with Child's B and C cirrhosis, the emergence of YMDD mutants led to increased CPT scores, despite IFN therapy (Fig. 2). Moreover, some patients with Child's B and C cirrhosis could not receive IFN therapy because of the risk of potentially life-threatening complications.[21,24] Therefore, the indications for lamivudine therapy in patients with Child's B and C cirrhosis must be carefully considered. These patients will need other antiviral agents with anti-HBV activity in the future. Recent studies suggest that adefovir dipivoxil and entecavir may effectively sup-

press YMDD mutants.[26,27] However, the efficacy and safety of these treatments in patients with HBV cirrhosis with YMDD mutants have not yet been established. In the near future, combination therapy with lamivudine and other anti-HBV agents may decrease the frequency of drug resistance and delay progression in patients with decompensated HBV cirrhosis.

In conclusion, our results suggest that lamivudine therapy improved the clinical course in some cirrhotic patients. However, the emergence rate of YMDD mutants in patients with HBV cirrhosis was higher than that in patients with chronic hepatitis. In patients with Child's B and C cirrhosis, the emergence of YMDD mutants sometimes led to worsening liver function. Therefore, the clinical course and laboratory data must be carefully checked prior to administration of lamivudine therapy in this group of patients. In future studies, combination therapy with two or more anti-HBV agents in patients with HBV cirrhosis should be evaluated in terms of clinical benefit and drug resistance.

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Hepatocellular carcinoma in noncirrhotic young adult patients with chronic hepatitis B viral infection

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Background. The aims of this study were to define the clinical characteristics of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) in young adult patients without cirrhosis and to evaluate the efficacy of interferon (IFN) therapy on HCC recurrence. ***Methods.*** Of 187 patients with HBV-related HCC treated at our hospital, 4 had no liver cirrhosis and were less than 30 years of age (10, 22, 23, and 26 years). ***Results.*** At the time of diagnosis of HCC, all cases had antibody to hepatitis B e antigen (anti-HBe) and histological staging of nontumorous liver was F0 or F1, i.e., low-grade hepatitis. The mothers of all 4 young adult patients with HCC had HBV-related liver disease. Three cases developed recurrence of HCC. In these patients, long-term intermittent IFN therapy after reresection of HCC resulted in long-term survival without recurrence for more than 3 years of follow-up. ***Conclusions.*** (1) Young adult patients with HCC are positive for anti-HBe, lack cirrhosis, and the route of infection seems to be mother-to-infant transmission. Transplacental transmission of HBV and HBV DNA integration into the cellular genomic DNA during fetal life is a possible explanation of HBV-related hepatocarcinogenesis in young adults; and (2) long-term IFN therapy seems to be useful for prevention of tumor recurrence after radical operation for HBV-related HCC.

Key words: young adult hepatocellular carcinoma, hepatitis B virus, transplacental transmission, HBV DNA integration, interferon, prevention

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide and is particularly prevalent in Southeast Asia. Several epidemiologic surveys have concluded that hepatitis B virus (HBV) and hepatitis C virus (HCV) are important factors in the development of HCC and that the majority of HCC developed in the presence of liver cirrhosis.[1–4] However, in chronic hepatitis B, the severity of fibrotic changes does not correlate with carcinogenesis, in contrast to chronic hepatitis C,[5–7] and we sometimes experience cases of HCC that do not correlate with any fibrotic stage of HBV infection.

HCC occurs mainly in adults with a peak age at 40–60 years, and in Japan, the average age of patients diagnosed with hepatitis B surface antigen (HBsAg)-positive HCC is reported to be 52 years.[8] However, we sometimes experience HBV-related HCC even in children and have noticed that HCC related to chronic HBV infection tends to occur much earlier in those infected in childhood than those infected in adulthood. In HBsAg-positive children, a high frequency of HBV integration is reported to occur at early stages of chronic infection, suggesting that the latency to the development of HCC is shorter than in adulthood.[9–12]

Interferon (IFN) is effective in patients with HBV-related chronic hepatitis and is known to reduce serum HBV DNA concentration, improve biochemical data, and consequently suppress disease progression to cirrhosis. Previous studies reported that IFN therapy successfully reduced hepatocellular carcinogenesis in patients with HBV-related cirrhosis and induced tumor regression with inoperable HCC.[13] On the other hand, in terms of HCV-related HCC, it is reported that long-term IFN therapy suppresses tumor recurrence after radical operation for HCC.[14]

The present study was designed to define the clinical characteristics of HCC in young adult noncirrhotic

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