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

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

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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 0.5 Meq/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 2 mm (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 first IFN therapy	
Effect of IFN retreatment	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 $50 \times 10^3/\mu l$ 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

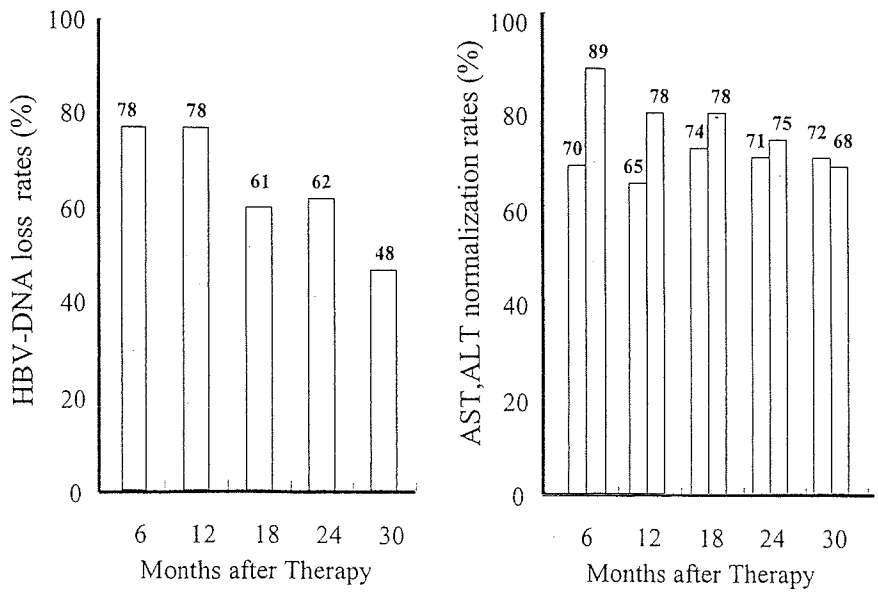

Fig. 1. A Hepatitis B virus (*HBV*)-DNA loss rates. **B** Alanine transaminase (*ALT*; *gray bars*) and aspartate transaminase (*AST*; *white bars*) normalization rates. The *numbers above each bar* represent the actual rate

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

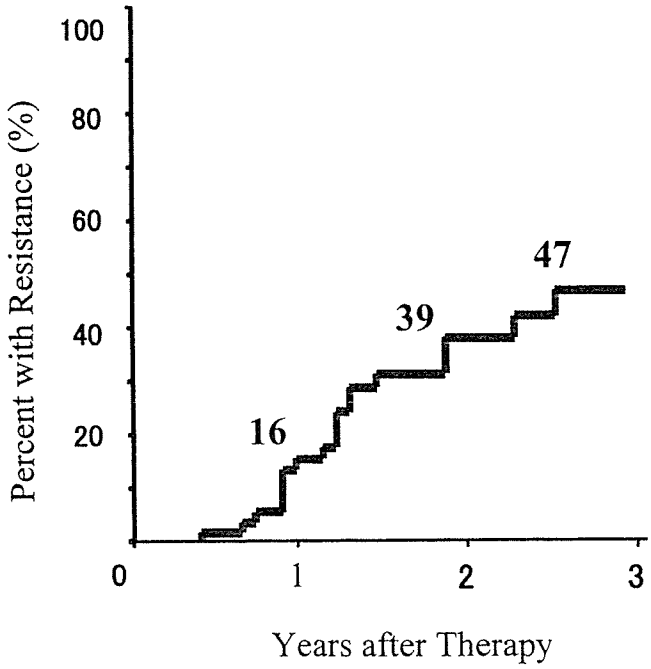

Fig. 2. Cumulative percentages of 54 patients who showed viral resistance during treatment with lamivudine (Kaplan-Meier analysis). *Numbers* represent the actual percentages for the indicated intervals

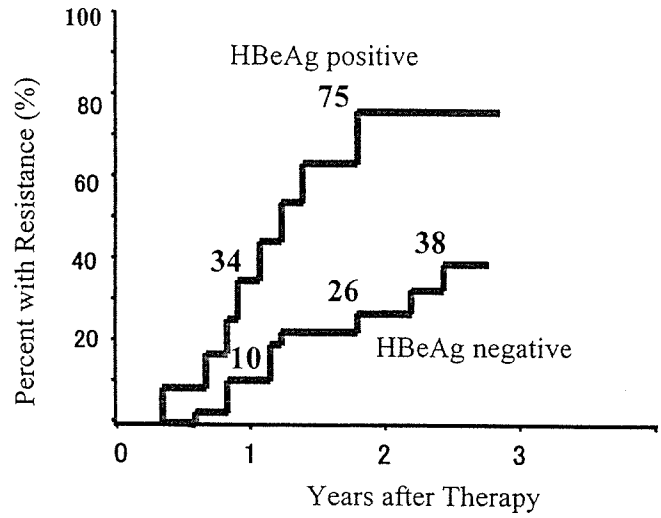

Fig. 3. Cumulative percentages of hepatitis B envelope antigen (*HBeAg*)-positive and -negative patients who showed viral resistance during treatment with lamivudine (Kaplan-Meier analysis). *Numbers* represent the actual percentages for the indicated intervals

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

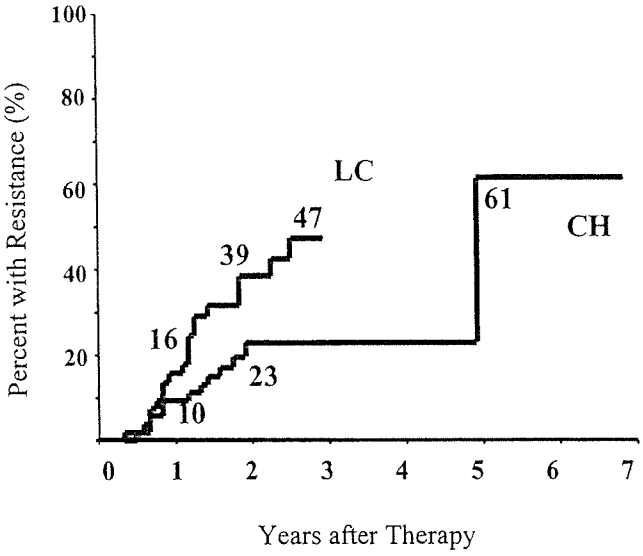

Fig. 4. Cumulative percentages of patients with liver cirrhosis (*LC*) and those with chronic hepatitis (*CH*) who showed viral resistance during treatment with lamivudine (Kaplan-Meier analysis). *Numbers* represent the actual percentages for the indicated intervals

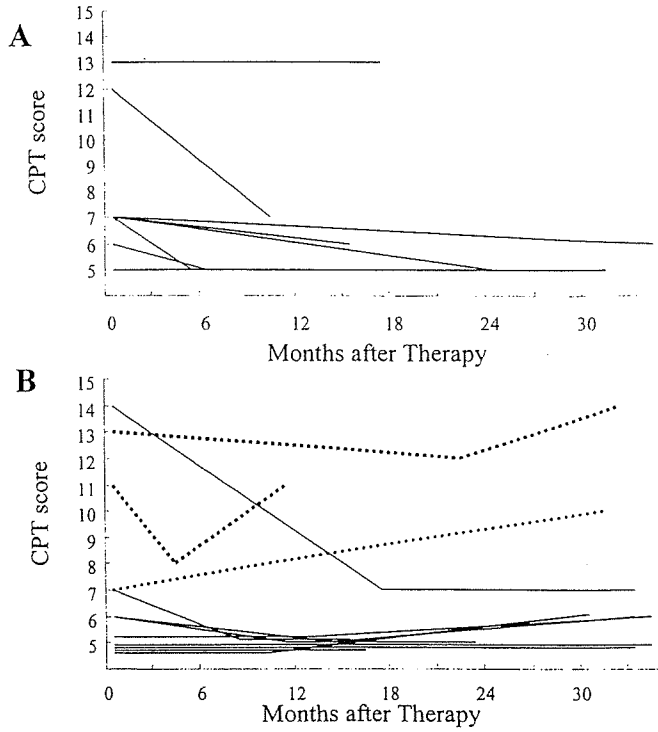

Fig. 5A,B. Individual serial Child-Pugh-Turcott (*CPT*) scores for patients treated with lamivudine. **A** Patients without breakthrough hepatitis (n = 9), and **B** patients with breakthrough hepatitis (n = 12). Clinical response was defined as a decrease in the CPT score of at least 2 points. Where 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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patients infected with HBV and to determine the effectiveness of IFN therapy in suppressing the rate of recurrence of HBV-related HCC in these patients. For this purpose, we compared young noncirrhotic patients with HCC with older noncirrhotic patients with HCC and also older cirrhotic patients with HCC.

Patients and methods

Patients

From August 1978 to October 2002, a total of 187 patients with chronic hepatitis B were treated for HCC at Toranomon Hospital, Tokyo, Japan. They included 155 males and 32 females, aged 10 to 80 years, with a median age of 52 years. Hepatitis B e antigen (HBeAg) was positive in 56 (30%) and anti-HBe in 98 (52%). All patients were negative for anti-HCV antibody. In terms of the incidence of HBV-related HCC by underlying liver tissue, HCC developed from liver cirrhosis cases in 166 (89%) of 187 cases and from chronic hepatitis cases in 21 (11%) cases.

Among these patients, all four patients who were less than 30 years of age had no cirrhotic liver disease. These four patients were labeled as cases of young adult HCC. They consisted of two males and two females ranging in age from 10 to 26 years with a median age of 22.5 years at the time of diagnosis of HCC.

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital, and a signed consent form was obtained from the subjects or their parents/relatives.

Blood tests

All four patients were positive for HBsAg as determined by hemagglutination, using commercially available kits (MyCell; Institute of Immunology, Tokyo, Japan), and HBeAg and anti-HBe as measured by radioimmunoassay (RIA; Abbot Diagnostics, Chicago, IL, USA). HBV-DNA was measured by branched DNA signal amplification technology (bDNA assay) (Chiron, Emeryville, CA, USA), transcription-mediated amplification and hybridization protect assay (TMA) (Chugai Diagnostics, Tokyo, Japan),[15] and Cobas Amplicor HBV Monitor Test (Amplicor) (Roche Diagnostics, Branchburg, NJ, USA). Genotyping of HBV was performed by an enzyme-linked immunosorbent assay (ELISA) kit (HBV Genotype EIA; Institute of Immunology, Tokyo, Japan) using monoclonal antibodies for the genotype-specific epitopes in the pre S2 region product.[16] Subgrouping of genotype B, Ba and Bj, was performed based on the method described by Sugauchi et al.[17] At the time of the diagnosis of HCC, background liver function was evaluated by indocyanine green retention rate at 15 min (ICG R15).

Liver histology

Liver tissues were obtained by surgical resection in one patient and by ultrasound-guided biopsies in two patients. Histological staging of the nontumorous liver tissue was based on the classification proposed by Desmet et al.[18] The remaining single patient did not undergo histopathological examination.

Family history

Information about the family history of HBV-related liver disease and the presence of HBsAg was obtained by asking patients and their family members about their past history.

Results

Incidence of HCC by age

Figure 1 shows the incidence of HBV-related HCC by age at diagnosis. For all 187 patients, the peak incidence of HCC associated with liver cirrhosis occurred in the 50–59 years age group while the peak incidence of HCC associated with chronic hepatitis occurred in the 40–49 years age group. Interestingly, all cases with HCC in the 10–19 and 20–29 years age groups had chronic hepatitis.

Comparison of clinicopathological profiles according to age and underlying liver tissue

We classified 187 HBV-related HCC cases into three categories depending on patient age (under or above 30

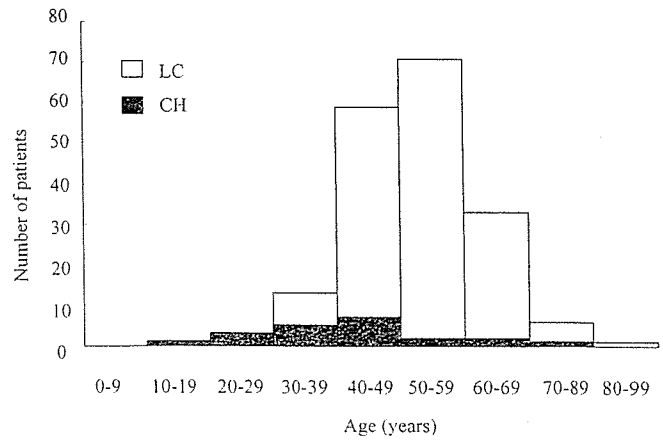

Fig. 1. Incidence of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) by age. *LC*, liver cirrhosis; *CH*, chronic hepatitis

Table 1. Comparison of profiles of hepatocellular carcinoma (HCC) patients without cirrhosis under 30 years of age, and those over 30 years of age with HCC and without cirrhosis or HCC with cirrhosis

	<30 years Cirrhosis (−) $n = 4$	≥30 years Cirrhosis (−) $n = 17$	≥30 years Cirrhosis (+) $n = 166$
Age (years)[a]	22 (10–26)	45 (36–45)	52 (32–80)
Sex (M:F)	2:2	15:2	138:28
eAg/eAb	0/4	3/12 (two sides, 2)	53/86 (two sides, 21) (unknown, 2)
HBV genotype A:B:C:D(E)	0:2:2:0	0:2:12:1 (not tested, 2)	0:6:148:0 (undetectable, 3) (not tested, 9)
Maternal HBV-related liver disease (%)	4 (100)	7 (41)	46 (28)

M, male; F, female; eAg, HBeAg; eAb, anti-HBe; HBV, hepatitis B virus
[a] Median value (range)

years), and presence or absence of liver cirrhosis. Table 1 provides a comparison of patient profiles. The proportion of females among the 4 young cases with young adult HCC (50%) was relatively high in comparison with the other two groups. Similarly, for the HBsAg genotype, the proportion of patients with genotype B was relatively higher in the test cases than in the other two groups.

In terms of the state of HBeAg/anti-HBe at the time of the diagnosis of HCC, all 4 test patients were positive for anti-HBe, whereas the proportions of cases positive for anti-HBe were 12 of 17 cases (71%) in the group without cirrhosis over 30 years of age and 86 of 166 cases (52%) in the group with cirrhosis over 30 years of age.

With regard to the family history, the mothers of all patients without cirrhosis under 30 years of age had HBV-related liver disease, including death from HCC in one, liver cirrhosis in two, and one asymptomatic carrier (Fig. 2). However, the incidence of maternal HBV-related liver disease in other groups tended to be lower; 7 of 17 cases (41%) in the group without cirrhosis over 30 years of age and 46 of 166 cases (28%) in the group with cirrhosis aged over 30 years of age.

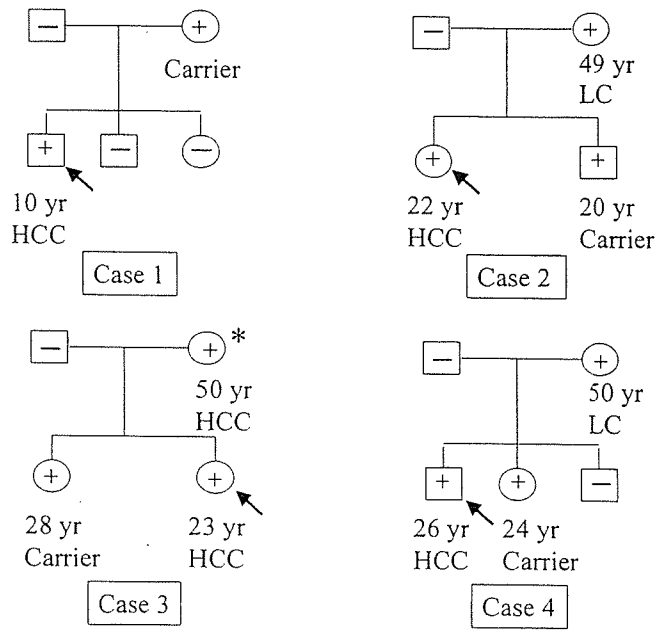

Fig. 2. Pedigree of four cases: +, positive HBsAg; −, negative HBsAg; *arrows*, HCC cases; *asterisk*, death

Clinical background of the four cases of young adult HCC

Table 2 summarizes the background characteristics of the four young adult HCC patients. Serum HBeAg was negative and anti-HBe was positive in all four cases. Distribution of HBV genotypes was two cases (50%) for genotype Bj and two cases (50%) for genotype C. HBV-DNA (b DNA assay) was measured in only one case (case 2) at the time of diagnosis of HCC, and serum HBV level was less than 0.7 mEq/ml. HBV-DNA was not measured in the other three cases because they were first diagnosed with HCC at other hospitals, and thus the HB viral loads of these patients were not clear. Laboratory tests revealed almost normal liver function based on the results of the ICG R15 test in all four patients.

Histopathological examination of the nontumorous liver was conducted in three of the four cases. In one case (case 1), there was almost normal architecture and

Table 2. Background of four cases with cirrhosis-unrelated HCC

Case	Sex	Age (years)	eAg/eAb	HBV genotype	Viral load bDNA assay (mEq/ml)	Histology	ICG (%)
1	M	10	−/+	Bj	NT	F0/A0	8
2	F	22	−/+	C	<0.7	NT	5
3	F	23	−/+	C	NT	F0/A0	6
4	M	26	−/+	Bj	NT	F1/A1	7

+, positive; −, negative; NT, not tested at the time of diagnosis of HCC; ICG, indocyanine green

Table 3. Clinical course of survival cases after HCC

Case	Treatment	Histological differentiation in last HCC	Viral load bDNA assay[a] (mEq/ml)	Intervals of interferon (IFN) administration[b] (years)	Survival period[c] (years)
1	TAE ×3 Operation ×3	Moderately	<0.7	3.3	>4.2
3	Operation ×2	Moderately–poorly	<0.7	3.1	>4.5
4	Operation ×2 TAE ×3	Moderately	<0.7	3.2	>3.5

TAE, transcatheter arterial embolization
[a] At the initiation of IFN therapy
[b] After completion of treatment for HCC, patients were administered 3 MU of IFN-α-2a twice a week
[c] Survival periods indicate time from the completion of treatment for HCC

no inflammation apart from mild irregularities in the size of hepatic cell nuclei. Case 3 also showed a normal liver architecture, very mild portal inflammation, and sparse focal necrosis in the lobular region. Case 4 showed a slight expansion and inflammation of the portal region and was diagnosed as chronic hepatitis (F1/A1).

Clinical course after HCC and efficacy of IFN therapy

At the time of diagnosis of HCC, case 2 had a large hepatoma that extended into both liver lobes with portal invasion, as evident by imaging studies including ultrasonography (US), computerized tomography (CT), and abdominal angiography. This patient underwent transcatheter arterial embolization (TAE) twice, but she died 4 months after the discovery of the tumor. Table 3 outlines the clinical course of the three surviving patients (cases 1, 3, and 4) after the diagnosis of HCC. Because they had recurrent HCC, they underwent surgical resection and TAE for HCC more than once. The histopathological differentiation determined for the last HCCs was not greater than moderately differentiated HCC in all three. After radical operation for HCC, each patient was treated with 3 million units (MU) of IFN-α-2a, twice a week, with the aim of preventing recurrence of HCC. Figure 3 shows the clinical course after the last therapy for HCC and the initiation of IFN therapy in these three cases. All patients showed stable low viral loads and normal liver function. As part of the follow-up monitoring for recurrence, imaging studies were performed every 3–4 months by using US or CT. These studies did not reveal recurrence of HCC during more than 3 years after the initiation of IFN therapy.

Discussion

Chronic infection with HBV is associated with increased risk for the development of cirrhosis and HCC. Based on the report of Ikeda et al.,[6] the incidence of progression of HBV-related chronic hepatitis to cirrhosis is 2.2% per year, whereas the incidence of carcinogenesis is 0.5% per year during the first 10 years and 1% thereafter. Using multivariate analysis, we also showed that the severity of the fibrotic stage in HBV-related chronic hepatitis was not associated with the development of HCC.[6] Another clinicopathological study of HCCs in chronic HBV carriers revealed that about 20%–50% of such patients do not have accompanying cirrhosis.[8]

With regard to the replicative state of HBV, after HBeAg to anti-HBe seroconversion associated with

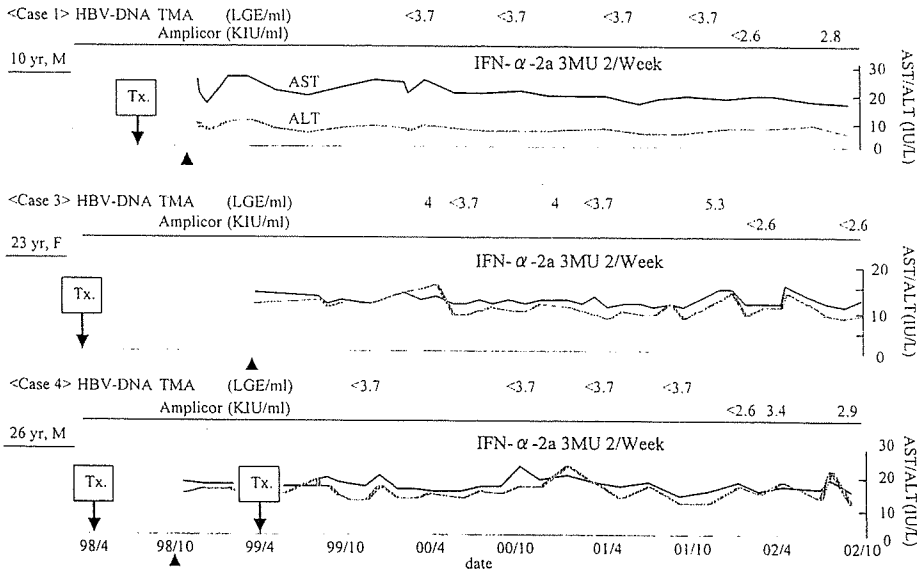

Fig. 3. Clinical course after radical surgery for last HCC. *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *Tx.*, therapy for HCC; *arrowheads*, time of first visit to our hospital

loss of viral replication and remission of disease activity, patients are usually asymptomatic and liver disease is inactive; however, some patients may progress to HCC.[19] In HCC, previous studies showed HBV DNA integration into the cellular genomic DNA.[4,10,11] Although the role of the integration in hepatocarcinogenesis is not clear at present, Dandri et al.[20] speculated that disturbances of repair of DNA damage, which occurred either on cellular or viral DNA with consecutive activation of recombination events, are responsible for the increased viral integration frequency.

HCC with HBV infection is sometimes found in very young patients, and the majorities of such patients have no cirrhosis and are negative for HBeAg but positive for anti-HBe, suggesting a nonreplicative state of HBV.[9] In these cases, HBV DNA integration is common. Because the high frequency of HBV DNA integration occurs at early stages of chronic infection, it has been suggested that the latent period of HCC after HBV infection is much shorter than in adult patients.[10-12]

In the present study, the characteristics of HBV-related HCC in young adult patients without cirrhosis were similar to those described in previous reports.[9-12] All cases were negative for HBeAg and positive for anti-HBe; the histopathological stage of nontumorous liver tissue was F0 or F1, representing mild hepatitis, at the time of diagnosis of HCC. Interestingly, family history of maternal transmission of hepatitis B virus was noted in all four cases. Unfortunately, we did not investigate the integration of HBV DNA into the tumorous and nontumorous liver tissues of these cases. Considering the above features and results of previous studies, we speculate that the development of HCC in our cases at a young age might be due to HBV DNA integration into the cellular genomic DNA during fetal life. It is very difficult to clearly explain why these four patients developed HCC at such a young age without cirrhosis. Although we cannot demonstrate any solid evidence, we hypothesize that at least in some cases, the development of HCC at a young age is related to transplacental transmission of HBV during intrauterine life from the infected mother and HBV DNA integration into the cellular genomic DNA during fetal life. Due to the HBV DNA integration in earlier stages of chronic infection, HCC developed within a short period at young age, as speculated by Chang et al.[9,11,12] and Goto et al.[10]

Previous studies reported a higher incidence of HCC in males than in females and that the rate of progression of HBV infection was faster in males than in females.[19] On the other hand, in terms of HBV genotypes, none of the reported HCC patients younger than 35 years of age had genotype B in Japan (genotype Bj), different from genotype B in Taiwan (genotype Ba), and genotype Bj is regarded to be associated with a relatively good prognosis.[21,22] Our results are different from those of the above reports; two of four patients are female and genotype B of HBV genotype, especially genotype Bj accounts for half of our patients. Furthermore, despite the relatively good prognosis reported in the these studies, our cases developed HCC early in life. Although the number of the young adult HCC was relatively small, these factors may indicate that HCC in young adults is a little different from HCC among elder patients.

HBV vaccine became available in 1980, and since 1986, initiatives have been taken to prevent mother-to-child transmission of HBV in Japan. Furthermore, since 1996, HBV vaccine has also been administered to

babies born to HBsAg-positive mothers without HBeAg. While the protection efficacy is high, 5%–10% of infants with intrauterine HBV infection caused by transplacental transmission develop the infection in spite of receiving HBV vaccine.[23] Although HBV vaccine against vertical transmission is clearly effective and may reduce the incidence of liver cirrhosis and HCC, HBV carriers who cannot be protected by HBV vaccine can develop HBV infection by transplacental transmission; in other words, the HBV DNA is already integrated into the host DNA at birth. These children should be carefully monitored for the development of HCC.

In a pilot study, we reported that long-term intermittent IFN therapy successfully reduced hepatocellular carcinogenesis in patients with HBV-related cirrhosis.[13] Our virological study showed that the role of IFN therapy from the point of view of cancer prevention was much more significant in patients with HBV DNA concentration ≧10 mEq, and hence we considered that IFN could suppress carcinogenesis in HBV-related cirrhosis through the suppression of HBV replication. In our study, all three surviving cases suffered repeated recurrence of HCC, and thus were considered a high-risk group for hepatocellular carcinogenesis. However, after the radical operation for the last HCC, and due to long-term intermittent IFN therapy, they showed good prognosis, survival, and no recurrence of more than 3 years, and also showed persistently low viral loads (by bDNA assay) and normal liver function during follow-up. Based on these observations, it seems that long-term intermittent IFN therapy could prevent the recurrence of HBV-related HCC and act as an antitumor agent.

Although we did not confirm the integration of HBV DNA into the cellular genomic DNA of these patients and have no evidence of transplacental transmission of HBV, these mechanisms remain speculative at this stage and further studies of a larger population sample are warranted.

In conclusion, we have demonstrated in the present study that (1) four young adult HCC patients were positive for anti-HBe, were free of liver cirrhosis, and in all the route of infection seemed 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 might be useful for prevention of tumor recurrence after radical operation for HBV-related HCC. To confirm these potential mechanisms, further long-term follow-up studies of large number of patients are necessary. Furthermore, prospective studies should examine the development of HCC in HBV carrier children who could not be protected by HBV vaccine, to confirm the presence of HBV DNA integration in cellular genomic DNA and also to investigate the host factors associated with early hepatocarcinogenesis, including oncogenes and suppressor oncogenes.

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