TABLE II. Characteristics of Two Patients Who Could Achieve HBsAg Clearance*

Patient	Age (year)	Sex	Genotype	Histology	Etiology of HBV infection[a]	Emergence of YMDD infection[a]	Emergence of BKH[c]	Treatment for BKH	Methods of lamivudine	Duration of lamivudine (year)
1	38	M	C	F2	Non-vertical	YVDD[b]	+	Interferon	Discontinuous	1.0
2	46	M	D	F1	Non-vertical	—	—	—	Discontinuous	1.1

*HBsAg clearance were determined by radioimmunoassay.
[a]Etiology of HBV infection in both patients was not vertical transmission from mother to infant.
[b]YMDD mutant type was continuous YVDD type, which tended to be detected in the relatively younger patients [Akuta et al., 2003c].
[c]BKH, breakthrough hepatitis.

lamivudine treatment, and HBsAg clearance at about 3 years after the cessation of lamivudine treatment was noted together with transient post-treatment ALT relapse.

In conclusion, HBsAg clearance occurred in two male patients with non-vertical transmission infection, who discontinued lamivudine therapy. One patient was a relatively young adult infected with HBV/C and developed breakthrough hepatitis and the other patient was infected with HBV/D but without YMDD mutant.

Development of Severe Breakthrough Hepatitis

Severe breakthrough hepatitis was defined as a rise in ALT level to ≥300 IU/L, accompanied by elevation of total bilirubin level to ≥3.0 mg/dl and coagulopathy with plasma prothrombin time of <75% of control activity. Severe breakthrough hepatitis occurred in only 5% of all patients (1/20), and in 7.7% of patients with the emergence of YMDD mutant (1/13). The patient was a male infected with HBV/C and developed severe breakthrough hepatitis at about 3 years after the start of lamivudine treatment, according to the mixed type of YMDD mutant [Akuta et al., 2003c]. He was suitably provided with combination therapy of lamivudine + IFN, which resulted in the stabilization of hepatitis. At the last visit, he was HBeAg negative and ALT normal level.

Development of Cirrhosis and HCC

None of the patients developed cirrhosis or HCC during follow-up and at end-point.

DISCUSSION

To our knowledge, this report provides the results of the longest follow-up study (median follow-up period of 8.5 years) of patients with chronic hepatitis B on the longest lamivudine treatment (median treatment period of 8.4 years). In our study, the rates of HBeAg negative, HBV-DNA undetectable, and ALT normal level at the last visit for the whole group tended to be and were significantly higher than those at the start of lamivudine. In particular, we could achieve very high rates (80% and 80%) of HBV-DNA undetectable and ALT normal level at the last visit, respectively, by including patients who received additional treatment for breakthrough hepatitis. Furthermore, these patients showed similar improvement at the last visit, irrespective of emergence of YMDD mutant and lamivudine therapy. In the present study of Japanese dominant HBV/C, the cumulative appearance rates of YMDD mutant and breakthrough hepatitis were also approximately similar to previous reports based on the shorter follow-up periods than this study [Lai et al., 1998; Liaw et al., 2000; Leung et al., 2001; Lok et al., 2003], and severe breakthrough hepatitis with icteric flare-up occurred in only 5% (only one infected with HBV/C). Furthermore, 80% of patients, who received suitable additional treatment for breakthrough hepatitis, were ALT normal level at the last visit. Hence, our results indicate that long-term lamivudine treatment also induces long-term favorable prognosis and is safe in patients with breakthrough hepatitis if suitable additional treatments are provided, to say nothing of the favorable prognosis in patients without YMDD mutant and breakthrough hepatitis. We agree with Lok et al. [2003] who provided data in support of the benefits of long-term lamivudine treatment [Akuta et al., 2004].

With regard to the treatment of breakthrough hepatitis, IFN and new nucleotide analogs (e.g., adefovir dipivoxil and entecavir) are reported to be effective in patients with YMDD mutant [Perrillo et al., 2000; Tassopoulos et al., 2001; Peters et al., 2002; Suzuki et al., 2002]. Especially, a recent report suggested that breakthrough hepatitis should be avoided, and that adefovir dipivoxil should be introduced when YMDD mutant is first detected or at the first sign of worsening hepatitis [Lok, 2004]. We agree with the recommendation of this report that other additional treatment for breakthrough hepatitis should be introduced earlier to achieve a more safe response to long-term lamivudine.

The benefits of continuous lamivudine after the emergence of YMDD mutant are still unclear. Previous studies showed that YMDD mutants are less replication-competent compared with the wild-type, and are associated with lower HBV-DNA levels compared with pretreatment HBV-DNA levels [Fu and Cheng, 1998; Lai et al., 1998; Melegari et al., 1998; Dienstag et al., 1999; Ling and Harrison, 1999; Ono-Nita et al., 1999; Leung, 2000]. We had reported that 3-year lamivudine therapy induced histopathological improvement regardless of the appearance of YMDD mutants, associated with breakthrough hepatitis, and suggested the benefit of long-term treatment [Suzuki et al., 2003]. In contrast, a recent study compared continuous lamivudine group with discontinuous group, and showed that there might be no benefit to continue treatment after emergence of

YMDD mutant based on comparison of the hepatitis flare rates, decompensation rates, HBeAg seroconversion rates, and HBV-DNA levels at a relatively short follow-up period [Liaw et al., 2004]. In the present study, we could not conclude whether it is beneficial to continue lamivudine therapy after emergence of YMDD mutant because we did not include a control group to compare between the continuous and discontinuous therapy, although patients with breakthrough hepatitis showed a favorable clinical course in response to additional treatment.

Recently, Liaw et al. [2003] reported that lamivudine treatment might suppress the disease progression and development of HCC in advanced chronic hepatitis B based on a median treatment duration of about 3 years. Ikeda et al. [2003] indicated that persistently low HBV-DNA might save patients from hepatocarcinogenesis in HBV-related cirrhosis, and our study also showed that the suppression of HBV-DNA levels with lamivudine also seems to suppress HCC. In our study of non-cirrhotic patients, the rate of HBV-DNA undetectable at the last visit was very high (80%) when we included five patients who received additional treatment for breakthrough hepatitis. This result supports the favorable prognosis of patients treated with long-term lamivudine who do not develop cirrhosis and HCC during follow-up. Furthermore, excluding cirrhotic patients as major risk factors for HCC might lead to a more favorable prognosis.

HBsAg clearance rates based on long-term lamivudine treatment remain inadequately defined at present. Previous reports from the United States indicated that the rate of HBsAg clearance was 23% in patients who discontinued lamivudine after HBeAg seroconversion and followed for up to 3 years [Dienstag et al., 2003]. Our results also indicated that HBsAg clearance occurred in only 10% of the whole group at a median follow-up of 8.5 years, but in contrast was noted in 33.3% of discontinuation patients and 0% in the continuous-treatment group. Thus, the discontinuation patients tended to achieve a higher rate of HBsAg clearance than continuous patients, and this result suggests that various factors, like virological rebound and/or immunological response after discontinuation of lamivudine, might affect HBsAg clearance. However, in this study of a small number of discontinuation patients, we could not conclude that HBsAg clearance could be achieved in the lamivudine discontinuation group, and further prospective studies should be performed. Interestingly, one patient, who could achieve HBsAg clearance regardless of breakthrough hepatitis, was a relatively young adult infected with HBV/C through non-vertical transmission; and the other patient infected with HBV/D, a rare type in Japan, was considered have acquired the disease through no-vertical transmission. These results suggest that these patients might have achieved HBsAg clearance with lamivudine based on the relatively short duration of HBV infection in comparison to vertical transmission and/or the different HBV genotype. To our knowledge, this is the first report that investigated the characteristics of HBsAg clearance during long-term lamivudine treatment. Further studies of a large group of patients are required to clarify whether the HBV genotype, duration of HBV infection, and method of lamivudine therapy influence HBsAg clearance following long-term lamivudine treatment.

In conclusion, the present study of Japanese patients with genotype C-dominant hepatitis B indicates that long-term lamivudine treatment is safe and induces long-term favorable prognosis, especially when a suitable additional treatment is used for breakthrough hepatitis. Further prospective studies are necessary to determine whether long-term lamivudine treatment improves long-term prognosis, including clearance of HBsAg and suppression of hepatocarcinogenesis.

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ORIGINAL ARTICLE

Long-term follow-up of interferon monotherapy in 454 consecutive naive patients infected with hepatitis C virus: Multi-course interferon therapy may reduce the risk of hepatocellular carcinoma and increase survival

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Abstract

Objective. The long-term effects of multi-course interferon (IFN) monotherapy in patients infected with hepatitis C virus (HCV) are still unclear. ***Material and methods.*** To evaluate the effects of multi-course IFN on hepatocarcinogenesis and survival, a follow-up study was conducted comprising 454 consecutively recruited non-cirrhotic naive patients infected with HCV, who had received IFN monotherapy between 1987 and 1992. The median follow-up was 11.3 years. ***Results.*** A sustained response (SR) after the first IFN was achieved by 152 patients (33.5%) (Group A). Of 302 patients (66.5%) with non-SR after the first IFN, 130 patients (28.6%) did not receive additional IFN (Group B), and the remaining 172 patients (37.9%) received multi-course IFN monotherapy (Group C). With regard to hepatocarcinogenesis and survival rates for liver-related deaths, Groups A and C both showed significantly better long-term clinical outcome than Group B ($p < 0.001$; log-rank test). Three independent factors were identified by multivariate analyses (fibrosis stage 3, Group B, and age ≥ 50) for all patients and two factors (fibrosis stage 3 and age ≥ 50) for Group C associated with hepatocarcinogenesis. With regard to hepatocarcinogenesis rates according to the mean alanine aminotransferase (ALAT) levels during the IFN-free period in Group C, significantly higher rates were noted in patients with ALAT levels above 1.5 $\times$ the upper normal limit (17.6%) than those below the limit (0%) ($p < 0.05$). ***Conclusions.*** Multi-course IFN monotherapy reduces the risk of hepatocarcinogenesis and increases survival, and low ALAT levels during the IFN-free period are associated with lower hepatocarcinogenesis rates in multi-course IFN.

Key Words: *HCV, hepatocellular carcinoma, interferon monotherapy, liver-related death, multi-course, survival analysis*

Introduction

Hepatitis C virus (HCV) usually causes chronic infection, which can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1–5]. In patients with HCV chronic hepatitis, treatment with interferon (IFN) can induce viral clearance and marked biochemical and histological improvement [6,7]. Recently, several studies based on single-course IFN monotherapy also showed that in patients receiving IFN therapy there was a reduced risk of development of HCC and liver-related death in comparison with untreated patients, especially in responders to the treatment [1,8–12]. However, it is still a matter of controversy whether or not the effect of IFN therapy is beneficial [13–21].

The response to IFN therapy varies among different HCV genotypes [22,23]. In Japan, about 70% of patients with chronic hepatitis C are infected with HCV genotype 1b, while about 25% are genotype 2a. Sustained response (SR) to IFN monotherapy is as low as 10 to 20% in genotype 1b infection [24–27]. We also encounter IFN-resistant patients infected with genotype 2a, even

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though SR is more than 60% in this group [28,29]. We previously reported that a second course of IFN monotherapy for IFN-resistant cases could increase the SR rates in patients who could not attain SR after the first course [30]. Thus, we reported an increase in the viral clearance rates as one of the short-term benefits of two or more courses (herein called multi-course) of IFN monotherapy. However, the long-term benefits of multi-course IFN monotherapy are still unclear, whether associated with SR or not.

The present study included 454 consecutive naive cases with chronic hepatitis C, in whom more than 10 years had elapsed since the induction of IFN monotherapy. The aims of the study were: 1) to evaluate the long-term efficacy of multi-course IFN therapy on hepatocarcinogenesis and survival, examined by analysis of the outcomes of single- and multi-courses of IFN; 2) to analyze the predictive factors associated with hepatocarcinogenesis, if any, in patients who received multi-course IFN therapy.

Material and methods

Patients

Among 573 consecutively recruited HCV-infected patients, in whom IFN monotherapy was induced between February 1987 and August 1992 at Toranomon Hospital, 454 were selected for the present study based on the following criteria: 1) patients naive to IFN monotherapy; 2) patients with chronic hepatitis, without cirrhosis or HCC, as confirmed by biopsy examination within 6 months of enrolment; 3) patients negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emerville, Calif., USA), and positive for HCV RNA qualitative analysis with PCR (nested polymerase chain reaction or Amplicor; Roche Diagnostic Systems, Calif., USA); 4) patients free of co-infection with human immunodeficiency virus; 5) patients not treated with antiviral or immunosuppressive agents within 6 months of enrolment; 6) lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake); 7) patients free of other types of hepatitis, including hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease; 8) patients without or with well-controlled diabetes; and 9) patients who consented to participate in the study.

With regard to the clinical features of 454 patients at the start of the first course of IFN monotherapy, there were 322 men and 132 women, aged 15–70 years with a median age of 48 years. The numbers of patients with fibrosis stages 1, 2, and 3 were 260, 160, and 34 patients, respectively. HCV genotypes were 1b in 313 patients and non-1b in 125, and the genotype in the remaining 16 patients was not determined. The median alanine aminotransferase (ALAT) level was 142 IU/l (range, 24–636 IU/l), and the median platelet count was $17.4 \times 10^4/\mu l$ (range, 7.4×10^4–$39.2 \times 10^4/\mu l$). The median viremia level was 2.7 Meq/ml (range, <0.5–67.1 Meq/ml). The median follow-up time was 11.3 years (range, 0.1–16.3 years).

Furthermore, at the first course of IFN monotherapy, 327 patients (72.0%) received IFN-α alone, 119 patients (26.2%) received IFN-β alone, while the remaining 8 patients (1.8%) received a combination of IFN-α and IFN-β. A median IFN dose per day of 6 million units (MU, range; 1–10 MU) was administered. As a whole, a median total dose of IFN of 526 MU (range; 10–3696 MU) was administered during a median period of 23.9 weeks (range; 0.6–205.4 weeks). Patients mainly received IFN monotherapy, including initial aggressive induction therapy (every day within 8 weeks, followed by three times per week).

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

Methods

The primary measure of efficacy of treatment was SR, defined as negative HCV RNA by qualitative analysis with PCR and normalization of transaminase levels (aspartate aminotransferase (ASAT), 11–38 IU/l; ALAT, 6–50 IU/l) at 24 weeks after cessation of IFN therapy. Patients who achieved SR after the first course of IFN monotherapy were classified as Group A. Patients who did not attain SR after the first course of IFN monotherapy were classified into two groups; based on whether they received other courses of IFN monotherapy or not. Patients who did not receive further courses of IFN monotherapy, because of concerns about adverse effects, lack of time for treatment, physicians' recommendation based on the emergence of depression and cardiopulmonary disease during and after the first course of IFN, or the lower levels of ALAT, were classified as Group B. Patients who received two or more courses of IFN monotherapy were classified as Group C.

Laboratory investigations

Blood samples were frozen at −80°C within 4 h of collection and were not thawed until used for testing. HCV genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of the NS5 region [31]. In all cases, HCV-RNA

viremia levels were measured by branched DNA assay version 2.0 (Chiron Corp) at commencement of therapy using frozen samples, and the results were expressed as 10^6 genomic equivalents per milliliter (Meq/ml). The lower limit of the assay was 0.5 Meq/ml. Samples with undetectable levels using this quantitative assay (<0.5 Meq/ml) were also evaluated by HCV-RNA qualitative analysis with PCR (nested polymerase chain reaction or Amplicor, Roche Diagnostic Systems) during and after therapy especially, and the results were expressed as positive or negative. The lower limit of the assay was 100 copies/ml.

Liver histopathological examination

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo) fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens for examination contained 6 or more portal areas. Histopathological diagnosis was made by an experienced liver pathologist (H.K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on histopathological assessment according to the scoring system devised by Desmet et al. [32].

Follow-up

Clinical and laboratory assessments were performed at least once every month before, during, and after treatment. Adverse effects were monitored clinically by means of careful interviews and medical examinations at least once every month. Patient compliance with treatment was evaluated with a questionnaire. Blood samples were also obtained at least once every month before, during, and after treatment, and were also analyzed for ALAT levels and HCV-RNA levels at various time-points.

Follow-up time represented the time from the start of the first course of IFN treatment until death, or until the last visit. During this time, we especially evaluated liver-related death, which included HCC, cholangiocellular carcinoma, liver failure, or esophageal variceal bleeding.

Diagnosis of hepatocellular carcinoma

Patients were examined for HCC by abdominal ultrasonography every 3 to 6 months. If HCC was suspected based on ultrasonographic results, additional procedures such as computed tomography, magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy, if necessary, were used to confirm the diagnosis.

Statistical analysis

The χ^2 test, Fisher's exact probability test, and the Mann-Whitney U-test were used to compare the background characteristics between groups. Multiple comparisons were examined using the Bonferroni test. The cumulative hepatocarcinogenesis and survival rates were calculated using the Kaplan-Meier technique, differences between survival curves were tested using the log-rank test. Statistical analyses of hepatocarcinogenesis and survival periods according to groups were calculated using the period from start of the first course of IFN monotherapy. The stepwise Cox regression analysis was used to determine independent predictive factors that were associated with hepatocarcinogenesis. We also calculated the odds ratios and 95% confidence intervals (95% CI). Potential predictive factors associated with hepatocarcinogenesis included the following 10 variables: age, gender, histological stage, HCV genotype, viremia level, serum ALAT, platelet count, total IFN dose, total IFN duration, and treatment group. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. Variables that reached statistical significance ($p<0.05$) or marginal significance ($p<0.10$) on univariate analysis were tested by the multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using SPSS software (SPSS Inc., Chicago, Ill., USA). All p-values of less than 0.05 by the two-tailed test were considered significant.

Results

Efficacy of IFN monotherapy

SR was achieved by 152 patients (33.5%) after the first course of IFN monotherapy (Group A). After the first course of IFN, 130 (28.6%) out of 302 (66.5%) non-SR patients did not receive a second course of IFN monotherapy (Group B), while the remaining 172 (37.9%) received two or more courses of IFN monotherapy (Group C). Of the 172 patients in Group C, 103 patients received two courses of IFN (30 of whom achieved SR), 51 patients received three courses (8 of whom achieved SR), 16 patients received four courses (4 of whom who achieved SR), and 2 patients received six courses (none achieved SR). Thus, 42 patients of Group C attained SR after multi-courses of IFN monotherapy.

In Groups A and B, the median total duration of IFN was 24.4 weeks (range, 4.0–205.4 weeks) and 23.4 (range, 2.9–90.0). The median total dose of IFN was 531 MU (range, 43–3696 MU) and 495 (range, 41–877). In the first, second, third, fourth, fifth, and sixth courses of IFN monotherapy in Group C, the median total durations of IFN were 23.9 weeks (range, 0.6–149.4 weeks), 24.0 (range, 1.3–313.7), 26.3 (range, 3.1–255.0), 29.6 (range, 3.9–86.3), 34.2 (range, 23.6–44.7), and 47.7 weeks (range, 25.3–70.1), respectively. In the first, second, third, fourth, fifth, and sixth courses of IFN monotherapy in Group C, the median total doses of IFN were 519 MU (range, 10–2399 MU), 573 (range, 29–4005), 525 (range, 28–3477), 566 (range, 81–1286), 555 (range, 402–708), and 690 (range, 180–1200), respectively. The median cumulative total durations and cumulative total doses, which represented the cumulative total duration and total dose of every course of every patient of Group C, were 58.5 weeks (range, 8.4–474.4 weeks) and 1380 MU (range, 340–5240 MU), respectively. The median periods free of IFN in Group C were 3.6 years (range, 0.1–7.7 years). Finally, the median dose of IFN per week in Groups A, B, and C were 21.8 MU/week (range, 6.7–42.0), 22.0 (range, 4.5–42.0), and 22.1 (range, 3.7–44.6), respectively.

Clinical features of patients of Groups A, B, and C

The clinical features of patients in Groups A, B, and C at the start of the first IFN monotherapy are summarized in Table I. The ages of patients of Group B were significantly higher than those of Group A ($p=0.001$; Bonferroni test) and Group C ($p=0.013$; Bonferroni test). Viremia levels and frequencies of genotype 1b in Group A were significantly lower than those in Group B ($p<0.0001$; Bonferroni test) and Group C ($p<0.0001$; Bonferroni test). Fibrosis stage of Group A was significantly milder than those of Group B ($p=0.005$; Bonferroni test) and Group C ($p=0.004$; Bonferroni test). There were no other significant differences in clinical features at the start of IFN therapy among the three groups.

Cumulative hepatocarcinogenesis rates in Groups A, B, and C

During the follow-up, 2 patients (1.3%), 23 (17.7%), and 19 (11.0%) developed HCC in Groups A, B, and C, respectively. In Groups A, B, and C, the cumulative hepatocarcinogenesis rates were 1.1, 15.0, 0.7% at the end of 5 years; and 2.2, 26.0, 9.0% at the end of 10 years, respectively. The rates were significantly different among the three groups ($p<0.0001$; log-rank test) (Figure 1). In particular, the rates in Group B were significantly higher than those in Group C ($p<0.0001$; log-rank test) and Group A ($p<0.0001$; log-rank test), and the rates in Group C were significantly higher than those in Group A ($p=0.0030$; log-rank test).

Cumulative survival rates for overall death and liver-related death in Groups A, B, and C

During the follow-up period, 2 patients (1.3%), 13 patients (10.0%), and 8 patients (4.7%) died in Groups A, B, and C, respectively. In Groups A, B, and C, the cumulative survival rates for overall death were 100, 95.0, and 100% at the end of 5 years; and 98.0, 78.0, and 96.0% at the end of 10 years, respectively. The rates were significantly different among the three groups ($p<0.0001$; log-rank test). Especially, the rate in Group B was significantly lower than those in Group C ($p=0.0003$; log-rank test) and Group A ($p<0.0001$; log-rank test). However, the rate in Group C was not significantly lower than that in Group A (no significance; log-rank test).

During the follow-up period, 0 patients (0%), 10 patients (7.7%), and 6 patients (3.5%) died of liver-

Table I. Patient characteristics at the start of first course of interferon monotherapy.

	Group A ($n=152$)	Group B ($n=130$)	Group C ($n=172$)
Sex (male/female)	113/39	81/49	128/44
Age (year)[a]	47 (15–64)[a]	52 (23–70)	47 (22–67)[c]
Viremia level (Meq/ml)[a]	1.4 (<0.5–45.0)	5.3 (<0.5–67.0)[d]	4.8 (<0.5–57.0)[e]
HCV genotype (1b/non 1b*/ND)	65/75/12	112/15/3[f]	136/35/1[g]
Fibrosis stage (F1/F2/F3)	103/45/4	62/60/8[h]	95/55/22[i]
ASAT (IU/l)[a]	81 (16–374)	74 (22–398)	75 (24–400)
ALAT (IU/l)[a]	151 (24–546)	120 (32–636)	140 (26–594)
Platelet count ($\times 10^4/\mu l$)[a]	18.7 (9.7–31.0)	17.1 (9.7–39.2)	16.5 (7.4–34.5)

[a](Median). [b]$p=0.001$, [c]$p=0.013$, compared with Group B by Bonferroni test. [d]$p<0.0001$, [e]$p<0.0001$.
[f]$p<0.0001$, [g]$p<0.0001$, [h]$p=0.005$, [i]$p=0.004$, compared with Group A by Bonferroni test.
*Non 1b of Group A (2a/2b/3b; 58/15/2), non 1b of Group B (2a/2b; 8/7), non 1b of Group C (2a/2b; 24/11).
Abbreviations: HCV = hepatitis C virus; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase.

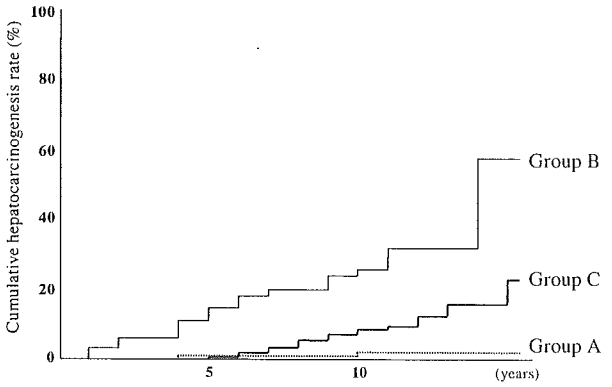

Figure 1. Cumulative hepatocarcinogenesis rates in the three study groups. The cumulative hepatocarcinogenesis rates were significantly different among the three groups ($p < 0.0001$; log-rank test). In particular, the rate in Group B was significantly higher than that in Group C ($p < 0.0001$; log-rank test) and that in Group A ($p < 0.0001$; log-rank test), and the rate in Group C was significantly higher than that in Group A ($p = 0.0030$; log-rank test). See text for the definition of the three groups.

related causes in Groups A, B, and C, respectively. All liver-related deaths were caused by liver cancer, and none died of hepatic failure without liver cancer. In Groups A, B, and C, the cumulative survival rates for liver-related deaths were 100, 96.6, and 100% at the end of 5 years; and 100, 83.7, and 97.8% at the end of 10 years, respectively (Figure 2). The rates were also significantly different among the three groups ($p < 0.0001$; log-rank test). In particular, the rate in Group B was significantly lower than that in Group C ($p = 0.0011$; log-rank test) and that in Group A ($p < 0.0001$; log-rank test), and the rate in Group C was significantly lower than that in Group A ($p = 0.0420$; log-rank test).

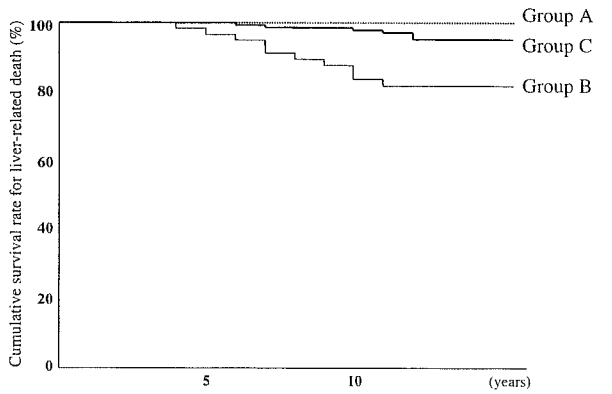

Figure 2. Cumulative survival rates for liver-related deaths. The cumulative survival rates for liver-related deaths were significantly different among the three groups ($p < 0.0001$; log-rank test). In particular, the rate in Group B was significantly lower than that in Group C ($p = 0.0011$; log-rank test) and that in Group A ($p < 0.0001$; log-rank test), and the rate in Group C was significantly lower than that in Group A ($p = 0.0420$; log-rank test).

Predictive factors associated with hepatocarcinogenesis by multivariate analysis

We then analyzed the data for the whole population sample to determine those factors that could predict hepatocarcinogenesis. Univariate analysis identified five parameters that tended to correlate, or significantly correlated with carcinogenesis. These included age ($p < 0.0001$), fibrosis stage ($p < 0.0001$), platelet count ($p < 0.0001$), total IFN dose ($p = 0.0691$), and Group ($p < 0.0001$). These factors were entered into a multivariate analysis, which then identified three parameters that independently influenced carcinogenesis; Group ($p = 0.008$), fibrosis stage ($p = 0.001$), and age ($p = 0.023$) (Table II).

Predictive factors associated with hepatocarcinogenesis in Group C by multivariate analysis

We also analyzed the data of 172 Group C patients to determine those factors that could predict hepatocarcinogenesis. Nine potential predictive factors associated with hepatocarcinogenesis were evaluated, excluding group of treatment. Three parameters were identified by univariate analysis that significantly correlated with hepatocarcinogenesis. These included age ($p = 0.0087$), fibrosis stage ($p < 0.0001$), and platelet count ($p = 0.0044$). All three factors were entered into a multivariate analysis, which in turn identified two parameters that independently influenced hepatocarcinogenesis, including fibrosis stage ($p < 0.0001$), and age ($p = 0.015$) (Table III).

Hepatocarcinogenesis rates according to fluctuation of ALAT levels at IFN-free period in Group C

In Group C, the hepatocarcinogenesis rates were also evaluated according to the mean ALAT levels at the IFN-free period. For this purpose, we selected 110 consecutive patients (64.0%) of Group C in whom ALAT levels were closely monitored. Thirty out of 110 patients (27.3%) achieved SR after multicourses of IFN monotherapy (SR group), and the

Table II. Factors associated with hepatocarcinogenesis in 454 patients, identified by multivariate analysis.

Factors	[Category]	Odds ratio (95% CI)	p-value
Fibrosis stage	1: F1, F2	1	
	2: F3	5.86 (2.18–15.7)	0.001
Group	1: A, C	1	
	2: B	3.17 (1.39–7.23)	0.008
Age (year)	1: <50	1	
	2: ≥50	2.63 (1.10–6.28)	0.023

Cox proportional hazards model.

Table III. Factors associated with hepatocarcinogenesis in 172 patients of Group C, identified by multivariate analysis.

Factors	[Category]	Odds ratio (95% CI)	*p*-value
Fibrosis stage	1: F1, F2	1	
	2: F3	10.9 (3.65–32.7)	<0.0001
Age (years)	1: <50	1	
	2: ≥50	3.87 (1.26–11.9)	0.015

Cox proportional hazards model.

remaining 80 patients (72.7%) could not attain an SR (NSR group).

Overall, the hepatocarcinogenesis rate in patients with ALAT levels below 1.5 × the upper limit of normal (<75 IU/l, normal for ALAT, 6–50 IU/l) was 0% (0 of 36 patients). The rates in those with ALAT from 1.5 × to 2 × (75–100 IU/l), from 2 × to 4 × (100–200 IU/l), and above 4 × (>200 IU/l) of the upper limit of normal were 23.8% (5 of 21 patients), 13.0% (6 of 46), and 28.6% (2 of 7), respectively (Table IV). In the NSR group, the hepatocarcinogenesis rate in patients with ALAT levels below 1.5 ×, from 1.5 × to 2 ×, from 2 × to 4 ×, and above 4 × were 0% (0 of 17 patients), 27.8% (5 of 18), 15.4% (6 of 39), and 33.3% (2 of 6), respectively. In the SR group, none of the patients developed HCC.

In conclusion, overall, the hepatocarcinogenesis rates in those patients with ALAT levels above 1.5 × (17.6%) the upper limit of normal were significantly higher than in those below 1.5 × (0%) of the upper limit of normal ($p=0.005$). In the NSR group, the hepatocarcinogenesis rates in those patients with ALAT levels above 1.5 × (20.6%) tended to be higher than in those with below 1.5 × (0%) ($p=0.059$).

Discussion

HCC is currently considered a very common malignancy and its incidence is increasing, in both Japan and the USA. Persistent HCV infection is a major risk factor for the development of HCC. About 80% of Japanese HCC patients are also diagnosed with HCV-associated cirrhosis or chronic hepatitis C [33]. Thus, HCV-related chronic liver disease is one of the major disorders affecting the national health of Japan, and prevention of HCC and improvement of survival remain the important issues in treatment for HCV-related chronic liver disease.

Several studies based on the single-course IFN monotherapy showed that IFN therapy reduced the risk of development of HCC and liver-related death in comparison with untreated patients and especially in responders to the treatment [1,8–12]. Furthermore, a recent report demonstrated that re-treatment with IFN at certain intervals also reduced the incidence of HCC in patients with chronic hepatitis C, even if eradication of HCV was not achieved by re-treatment [34]. Apart from defining the suppressive action of the multi-course IFN treatment on HCC, the same study, however, indicated that the long-term benefits of such treatment were still inadequately defined, probably because the survival rate and the incidence of hepatocarcinogenesis based on fluctuation of ALAT at the IFN-free period were not analyzed. Hence, in our study, we evaluated 454 consecutive naive cases with chronic hepatitis C, in whom more than 10 years had elapsed since the induction of IFN monotherapy at our hospital (i.e. a longer follow-up period than that in the previous report [34]), to examine the influence of multi-course IFN on hepatocarcinogenesis and survival.

Our group previously reported that the cumulative hepatocellular carcinogenesis rates in HCV patients of untreated and SR groups for IFN monotherapy were 4.8 and 1.4% at the end of 5 years, and 12.4% and 1.4% at the end of 10 years, respectively [10]. In this study, the hepatocarcinogenesis rates were low in the SR group who received a single-course IFN (Group A), which were 1.1 and 2.2% at the end of 5 and 10 years, respectively. However, the hepatocarcinogenesis rates in patients of Group B who received a single-course IFN but did not show SR (15.0 and 26.0% at the end of 5 and 10 years, respectively) were higher than those of untreated patients described in our previous study [10]. This discrepancy between our results and the previous report may be due to differences in patients' backgrounds, as patients of the present study had higher ALAT levels and more progressive stages of fibrosis, which are known risk factors for

Table IV. Hepatocarcinogenesis rates according to the fluctuation of ALAT levels at the IFN-free period in Group C.

	ALAT levels (IU/l)[a]			
	<75	75–100	100–200	>200
Hepatocarcinogenesis rate (%)	0%	23.8%	13.0%	28.6%
(Number)	(0/36)	(5/21)	(6/46)	(2/7)

[a]Normal level of ALAT, 6–50 IU/l.
Abbreviations: ALAT = alanine aminotransferase; IFN = interferon.

HCC [11,33,35,36], than those of the previous study.

With regard to multi-course IFN, Hino et al. [34] recently reported that re-treatment reduced the incidence of HCC, although the follow-up duration of their population sample was less than 10 years. Our study, based on a longer follow-up period of 10 years or more, also indicated that hepatocarcinogenesis rate in the multi-course IFN (Group C) was lower than that in the non-SR group after a single course of IFN (Group B). Considered together, we conclude that the hepatocarcinogenesis rates in Groups A and C were significantly lower than those in Group B, indicating that HCV-RNA eradication at an early stage, or multi-course IFN irrespective of SR are important for the suppression of hepatocarcinogenesis. To our knowledge, our study is the first to report the hepatocarcinogenesis rates for a follow-up period of 10 years or more in multi-course IFN.

In a previous study from our laboratory, we reported that the cancer-suppressive activity of single-course IFN monotherapy in patients with HCV-RNA eradication was similar to that in patients with ALAT normalization without HCV-RNA elimination [10]. Other studies also indicated a higher incidence and more rapid development of HCC in HCV patients with high ALAT levels [35,36]. Collectively, these results suggest that the carcinogenic process in patients with chronic hepatitis C is enhanced by high levels and fluctuations of ALAT, and indicate a close relationship between suppression of inflammatory necrosis of hepatocytes and lower incidence of HCC in patients with HCV-associated chronic liver disease.

Our results of multi-course IFN therapy are the first to show a 0% hepatocarcinogenesis rate in patients with ALAT levels below 75 IU/l at the IFN-free periods, emphasizing the importance of keeping low ALAT levels at such periods with respect to suppression of hepatocarcinogenesis. Thus, patients who fail to achieve SR after single-course IFN should receive multi-course IFN at the time of ALAT relapse, at certain intervals. Based on our results and those of Okanoue et al. [37], who demonstrated an increased incidence of HCC in 5 years or more after IFN therapy in transient biochemical responders, it is important to normalize ALAT levels by multi-course IFN at certain intervals.

Previous studies have shown that gender, age, fibrosis stage, and IFN regimen are important pretreatment predictors of hepatocarcinogenesis [10,11,34]. In the present study, higher age and a more progressive fibrosis stage were associated with higher hepatocarcinogenesis rates in the whole population sample and in Group C. Furthermore, our analysis of IFN treatment-related factors showed that Group B (non-SR after single-course IFN) was also a risk factor for hepatocarcinogenesis. This result is almost similar to that of a previous study, which identified the number of courses of IFN as a risk factor for HCC [34].

Previous studies indicated that the liver-related overall mortality rates of HCV patients were 57–94%, emphasizing the need for improvement in survival of patients with chronic hepatitis C with or without cirrhosis [1,10,12,18–21,38]. Our results also indicated that the proportion of liver-related overall deaths was 69.6% (16 of 23 patients). Our study is the first to report the survival rates for overall death and liver-related death in multi-course IFN therapy (Group C). The results indicated that overall and liver-related deaths in Groups A and C were significantly lower than those in Group B, suggesting that HCV-RNA eradication at an early stage or multi-course IFN irrespective of SR is also important for reducing liver-related deaths, like the suppression of hepatocarcinogenesis.

The aims of IFN therapy for chronic hepatitis C include the reduction of the risk of development of HCC and liver-related death by viral clearance, and when viral clearance could not be achieved, then by ALAT normalization without viral clearance. The reported post-IFN monotherapy viral clearance rate for genotype 1b (the most major type in Japan) is as low as 10 to 20% [24,26,27]. The antiviral efficacy of IFN has improved recently following combination with ribavirin [39–41] and the use of pegylated IFN [42–45], but these modifications also could not achieve a sufficient SR (SR is still $<50\%$ in patients with genotype 1 with higher pretreatment viral loads). Furthermore, the combination therapy is sometimes associated with serious adverse effects such as hemolytic anemia [46]. Hence, the development of more effective and safe IFN regimens is needed.

In conclusion, our retrospective study indicates that multi-course IFN monotherapy reduces the risk of hepatocarcinogenesis and increases survival. Large-scale prospective studies should be conducted in the future to confirm this finding.

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Persistence of Acute Infection With Hepatitis B Virus Genotype A and Treatment in Japan

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Among the 97 adult patients with acute hepatitis B who were admitted to the Toranomon Hospital in Metropolitan Tokyo during 28 years from 1976 to 2003, 31 (32%) were infected with hepatitis B virus (HBV) genotype A, nine (9%) with genotype B, 44 (45%) with genotype C, one (1%) each with genotypes E and F. HBV in the remaining 11 (11%) patients were untypeable. All the 31 patients with acute hepatitis B caused by HBV genotype A infection were male with a median age of 31 years, and 16 (52%) contracted infection through extramarital sexual contacts. The baseline HBV DNA level was higher in the seven (23%) patients in whom infection with HBV genotype A persisted than the remaining 24 (77%) with spontaneous resolution (median: >8.7 vs. 6.0 log genome equivalents/ml, $P=0.004$). Persistent infection was more frequent in patients with maximum alanine aminotransferase <500 IU/L than ≥500 IU/L (83% [5/6] vs. 4% [1/25], $P=0.0001$). Of the six patients with persistent HBV genotype A infection who received interferon and/or lamivuidine for treatment of chronic active hepatitis, three (50%) responded with the loss of hepatitis B e antigen (HBeAg); hepatitis B surface antigen (HBsAg) was cleared from serum in one patient who received interferon and lamivudine in sequence. HBV genotype A persisted along with HBeAg in the remaining three patients given antiviral therapy as well as another who was not treated. In conclusion, infection with HBV genotype A prevails in patients with acute hepatitis B in Japan where genotypes B and C are common, is often contracted sexually (16/31 [52%]) and tends to persist (7/31 [23%]). Infection was cleared in only one of the six (17%) patients who received antiviral therapy. ***J. Med. Virol. 76:33–39, 2005.*** © 2005 Wiley-Liss, Inc.

KEY WORDS: acute hepatitis; chronic hepatitis; genotypes; hepatitis B virus; interferon; lamivudine

INTRODUCTION

Approximately 350 million people are infected persistently with hepatitis B virus (HBV) throughout the world [Lee, 1997], and most reside in Asia and Africa. There are eight genotypes of HBV, defined by a sequence divergence in the entire genome exceeding 8%, and are designated by capital alphabet letters from A to H in the order of discovery [Okamoto et al., 1988; Norder et al., 1992; Stuyver et al., 2000; Arauz-Ruiz et al., 2002]. Genotypes of HBV have distinct geographical distributions [Magnius and Norder, 1995; Lindh et al., 1997; Miyakawa and Mizokami, 2003], and are associated with the severity of liver disease and responses to antiviral treatment [Chu and Lok, 2002; Kao, 2002; Miyakawa and Mizokami, 2003].

In countries highly endemic for HBV, such as China and Africa, the carrier state is established mainly through horizontal transmission during infancy [Botha et al., 1984; Yao, 1996]. In Europe and the United States where the prevalence of HBV is low, by contrast, persistent infection occurs predominantly by infection in

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adults. Japan is exceptional in that perinatal transmission from infected mothers had been the main route of persistent HBV infection [Okada et al., 1976], before the national immunoprophylaxis program for babies born to carrier mothers was launched in 1986 [Noto et al., 2003].

In Japan, genotypes B and C prevail and together they account for more than 95% of persistent HBV infection [Orito et al., 2001; Kobayashi et al., 2002, 2003]. Acute infection with HBV genotype A keeps increasing, however, particularly in men with extramarital sexual contacts [Kobayashi et al., 2002, 2003, 2004; Ogawa et al., 2002]. Infection with HBV genotype A in the adult has a high propensity to become chronic, which would contribute to the persistent carrier state in Western countries where the perinatal or childhood transmission of HBV is rare [Heijtink et al., 1999; Lindh et al., 2000].

During 28 years from 1976 to 2003, 31 patients were diagnosed with acute hepatitis B who were infected with HBV genotype A at the Toranomon Hospital in the Metropolitan Tokyo. The infection persisted in seven (23%) patients accompanied by biopsy-proven chronic hepatitis B. Their clinical course were followed with a special reference to the response to antiviral treatment.

MATERIALS AND METHODS

Patients With Acute Hepatitis B

During 28 years from August 1976 through September 2003, 97 patients were diagnosed with acute hepatitis B at the Department of Gastroenterology at the Toranomon Hospital in Metropolitan Tokyo. Genotypes of HBV were A in 31 (32%) of them, B in nine (9%), C in 44 (45%), E and F in one (1%) each, while they were untypeable in the remaining 11 (11%). The 31 patients with acute hepatitis B caused by infection with HBV genotype A were followed clinically and examined virologically. Seven of them (23%) developed persistent HBV infection accompanied by chronic hepatitis B, and six received antiviral therapy and evaluated for the response. All the patients possessed IgM antibody to hepatitis B core (anti-HBc) in high titers, but they were negative for antibody to hepatitis delta virus, IgM antibody against hepatitis A virus or antibody to hepatitis C virus. A number of these patients have been reported previously with respect to clinical features [Kobayashi et al., 2003], perpetuation of acute infection [Kobayashi et al., 2002] and shifts of HBV genotypes with time [Kobayashi et al., 2004]. The study protocol conformed to the 1975 Declaration of Helsinki, and was approved by the Ethic Committee of Toranomon Hospital. An informed consent for this study was obtained from each patient.

Serological Markers of HBV Infection

Hepatitis B surface antigen (HBsAg) was determined by hemagglutination (MyCell, Institute of Immunology Co., Ltd., Tokyo, Japan) and hepatitis B e antigen (HBeAg) by enzyme-linked immunosorbent assay (ELISA) with commercial kits (ELISA, F-HBe; Kokusai Diagnostic, Kobe, Japan). Anti-HBc of IgM class was determined by radioimmunoassay (HBc-antiM RIA, Dinabot, IL). HBV DNA was determined by transcription-mediated amplification and hybridization assay (TMA; Chugai Diagnostics, Tokyo, Japan) and the results were expressed as log genome equivalents (LGE) per milliliter of serum, over a detection range from 3.7 to 8.6 LGE/ml.

Genotypes of HBV

The six major genotypes (A–F) were determined by ELISA by the combination of epitopes on preS2-region products by monoclonal antibodies which is specific for each of them [Usuda et al., 1999, 2000] by commercial assay kits (HBV GENOTYPE EIA; Institute of Immunology, Co., Ltd., Tokyo, Japan). Genotype G was determined by the preS2 serotype for genotype D and HBsAg serotype adw [Kato et al., 2001]; the combination is specific for this genotype.

Genetic Subgroups of Genotype A

Subgroups of genotype A designated Ae prevalent in Europe and Aa frequent in Africa, as well as Asia [Sugauchi et al., 2004] that correspond to subgroup A′ reported originally by Bowyer et al. [1997], were determined by the nucleotide (nt) sequence in the S gene specific for each of them [Sugauchi et al., 2003]. Briefly, nucleic acids were extracted from serum and a sequence of the large S gene was amplified by polymerase chain reaction (PCR) with nested primers. The first-round PCR was carried out with BGF1 (sense, 5′-CTG TGG AAG GCT GGC ATT CT-3′ [nt 2,757–2,776]) and BGR2 (antisense, 5′-GGC AGG ATA GCC GCA TTG TG-3′ [nt 1,050–1,079]) primers, and the second-round PCR with PLF5Bm (sense, 5′-TGT GGA TCC TGC ACC GAA CAT GGA GAA-3′ [nt 136–162]) and BR112 (antisense, 5′-TTC CGT CGA CAT ATC CCA TGA AGT TAA GGG A-3′ [nt 865–895]) as well as BGF5 (sense, 5′-TGC GGG TCA CCA TAT TCT TG-3′ [nt 2,811–2,830]) and BGR6 (antisense, 5′-AGA AGT CCA CCA CGA GTC TA-3′ [nt 249–268]) for 35 cycles each (94°C, 1 min [5 min in the first cycle]; 53°C, 2 min; and 72°C, 3 min [7 min in the last cycle]). The amplification products were run on gel electrophoresis and stained with BIG Dye (Applied Biosystems, CA). The they were purified by Qiquick PC purification kit (Qiagen, Hilden, Germany), and sequenced in AGI Prism 310 Genetic Analyzer (Applied Biosystems).

Antiviral and Other Treatment

Patients in whom HBV infection persisted and with chronic hepatitis diagnosed by liver biopsy, received interferon (IFN) or lamivudine, or both in sequence. Natural IFN-α (Smiferon; Sumitomo Pharmaceutical Co., Ltd., Tokyo, Japan) or IFN-β (Feron, Toray Co., Ltd., Tokyo, Japan) in a dose of 3 or 6 mega units (MU) was injected subcutaneously two or three times in week (tiw) for up to 1 year with or without induction by 6 MU

daily for 8 weeks. Lamivudine (Glaxo-Welcome, Greenford, UK) was given orally at a daily dose of 100 mg until HBeAg was lost from serum in responders, and continued indefinitely in non-responders who failed to achieve HBeAg seroconversion. The response to antiviral treatment was defined by the loss of HBeAg from serum accompanied by normalization of ALT levels and clearance of HBV DNA determined by the TMA method with the detection limit of 3.7 LGE/ml.

Some patients had received glycyrrhizin either intravenously (Stronger Neo-Minophagen C [SNMC]; Minophagen Pharmaceutical Co., Ltd., Tokyo, Japan) or orally (GLYCYRON Tab; Minophagen Pharmaceutical Co., Ltd.), with or without oral ursodeoxycholic acid (UDCA; Mitsubishi Welpharmar Co., Ltd., Tokyo, Japan), before they were referred to the Toranomon Hospital.

Statistical Analysis

Categorical variables were compared between groups by the χ^2-test or Fisher's exact test, and non-categorical variables by the Mann–Whitney U-test.

RESULTS

Patients With Acute Hepatitis B Infected With HBV Genotype A

Infection resolved spontaneously in 24 of the 31 patients with acute hepatitis B who were infected with HBV genotype A, while it persisted in the remaining seven (23%) patients none of whom carried human immunodeficiency virus type 1. The persistence of acute infection tended to be more frequent in patients infected with HBV genotype A than those with genotype B (1/9 [11%]), or C (3/42 [7%]); one each patient infected with genotypes E and F cleared infection.

Table I compares demographic, clinical, and virological characteristics between patients in whom HBV infection did and did not persist. All the seven patients with chronic HBV infection were men, and tested negative for HBsAg in serum before they developed acute hepatitis; HBsAg persisted in them during 6 months or longer after they first tested positive for it. Of the 31 patients with acute infection with HBV genotype A, 16 (52%) confided having had extramarital sexual contacts. Homosexual activities were experienced somewhat more frequently in patients with than without persistent HBV infection (4/7 [57%] vs. 5/24 [22%]).

The maximum median ALT level was significantly lower (234 vs. 1,836 IU/L, $P = 0.0001$), while median HBV DNA level was significantly higher (median: >8.7 vs. 6.0 LGE/ml, $P = 0.004$) in patients in whom HBV genotype A infection persisted than in those who cleared it (Table I). None of the eight patients with HBV DNA <5 LGE/ml developed persistent infection. Infection persisted significantly more often in patients with the maximum ALT <500 IU/L than ≥500 IU/L (5/6 [83%] vs. 1/25 [4%], $P = 0.0001$).

Genetic subgroup of genotype A was Ae (the original European type) in all the 20 patients for whom subgrouping was possible. None of them were infected with HBV of subgroup Aa (Asian/African type corresponding to A′ of Bowyer et al. [1997]). Of the seven patients in whom infection persisted, five were infected with HBV of subgroup Ae; subgrouping was not feasible in the remaining two (Cases 2 and 4 in Table II).

Clinical Courses of the Seven Patients in Whom Infection With HBV Genotype A Persisted

Of the seven patients in whom infection with HBV genotype A persisted, six received treatment with IFN and/or lamivudine after transfer to Tornomon Hospital (Table II). They all had chronic hepatitis in the first liver biopsy; it was undertaken before treatment in five (71%). Three of them (50%) responded to treatment with the clearance of HBeAg from serum, normalization of ALT levels and loss of HBV DNA determined by the TMA method with the detection limit of 3.7 LGE/ml. In the remaining four patients, including the single one (Case 7) who did not receive antiviral treatment due to the absence of active hepatitis, infection with HBV genotype A persisted along with HBeAg and fluctuating ALT levels in serum.

Figure 1 depicts clinical courses of the three patients who responded to antiviral treatments. Case 1 received

TABLE I. Baseline Characteristics of Patients With Acute Hepatitis Induced by HBV Genotype A in Whom Hepatitis Persisted or Resolved

Features	Persisted (n = 7)	Resolved (n = 24)	Differences
Male	7 (100%)	24 (100%)	NS[c]
Age (years)[a]	26 (21–54)	33 (25–56)	NS
Sexual transmission	5 (71%)	11 (48%)	NS
Homosexual	4 (57%)	5 (22%)	
Heterosexual	1 (13%)	6 (26%)	
Maximum ALT (IU/L)[a]	234 (143–774)	1,836 (46–3,300)	$P = 0.0001$
HBsAg titer (2^N)[b]	11 (11 to ≥13)	11 (8 to ≥13)	NS
HBeAg-positive	7 (100%)	23 (96%)	NS
HBV DNA (LGE/ml)	>8.7 (6.3 to >8.7)	6.0 (<2.6 to >8.7)	$P = 0.004$

[a]Median values are shown with the range in parentheses.
[b]Determined by the hemagglutination assay on serial twofold dilutions of serum.
[c]Not significant.

TABLE II. Routes of Infection, Liver Histology, and Treatment Outcomes of the Seven Patients in Whom Infection With HBV Genotype A Persisted

Case no.	Age/sex	Sexual contacts[a]	Liver histology[b]	Treatment[c]	HBeAg lost	HBsAg lost
1	54/M	None	F1/A2	Lam	Yes	Yes
2	43/M	Hetero	F2/A1	IFN	Yes	No
3	46/M	None	F4/A2	IFN	Yes	No
4	21/M	Homo	F1/A1	IFN/Lam	No	No
5	24/M	Homo	F1/A2	Lam	No	No
6	28/M	Homo	F1/A1	IFN/Lam	No	No
7	21/M	Homo	F1/A1	None	No	No

[a]Extramarital sexual contacts in which infection with HBV genotype A was implicated.
[b]Pathology of the liver in the first biopsy; F, fibrosis stage; A, activity grade.
[c]Treatment received after admission to the Department of Gastroenterology in Toranomon Hospital; IFN, interferon; Lam, lamivudine.

lamivudine 3 months after the admission to our hospital. He lost both HBeAg and HBsAg, respectively, within 83 and 90 days on lamivudine; it was withdrawn at the disappearance of serum HBsAg. ALT levels normalized after the loss of HBeAg and HBsAg from serum.

Cases 2 and 3 did not receive treatment during 4 and 2 years, respectively, after they visited the hospital. They both responded to IFN 3 MU three times in week and lost HBeAg from serum, along with the disappearance of HBV DNA and normalization of ALT levels. They did not, however, clear HBsAg from serum. Cases 1–3 had received oral (Glycyron) or intravenous (SNMC) glycyrrhizin with or without ulsodeoxicholic acid (UDCA) while they were admitted to other institutions before referral to the Toranomon Hospital.

Clinical courses of the three patients who did not respond to antiviral therapies (Cases 4–6) are illustrated in Figure 2, along with that of a single patient who did not receive treatment (Case 7). Case 6 had received intravenous glycyrrhizin (SNMC) before his

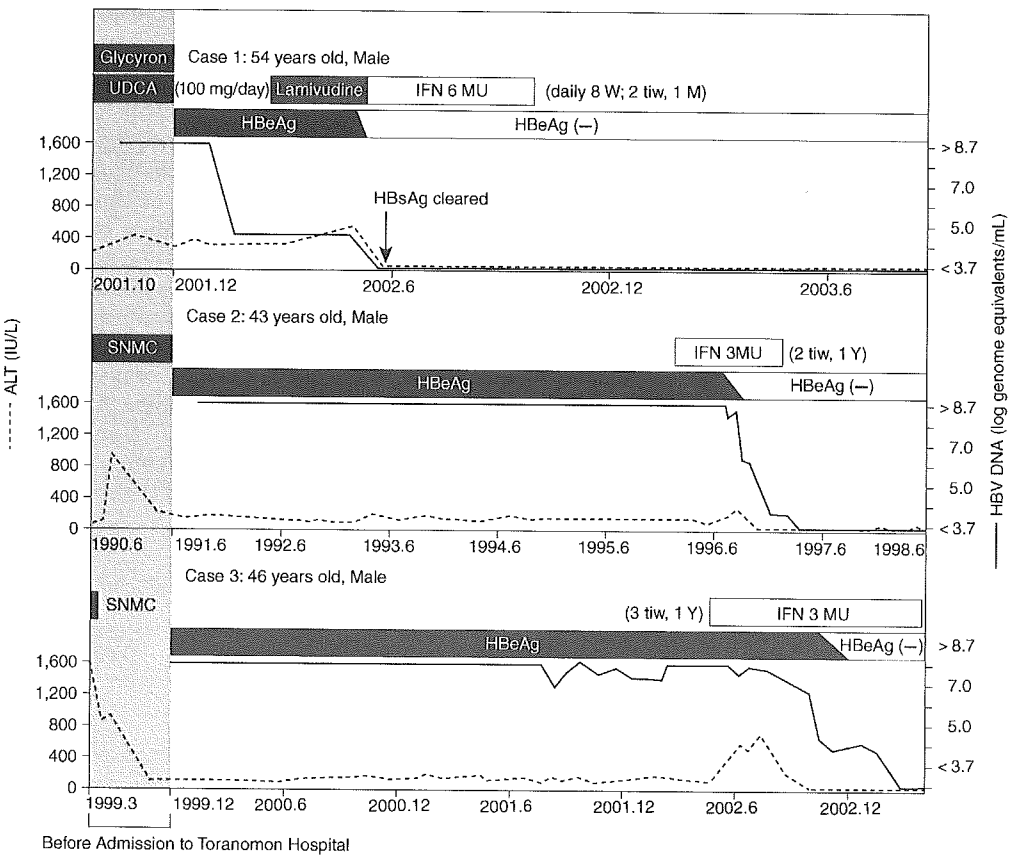

Fig. 1. Clinical courses of the three patients infected with HBV genotype A who responded to antiviral treatment with the loss of HBeAg. Courses and treatment they received before admission to Gastroenterology Department in Tornomon Hospital are shown in shaded areas on the **left**. The patient in Cases 1 lost HBsAg after treatment with lamivudine at time points indicated by arrows. IFN, interferon; SNMC, Stronger Neo-Minophagen C.

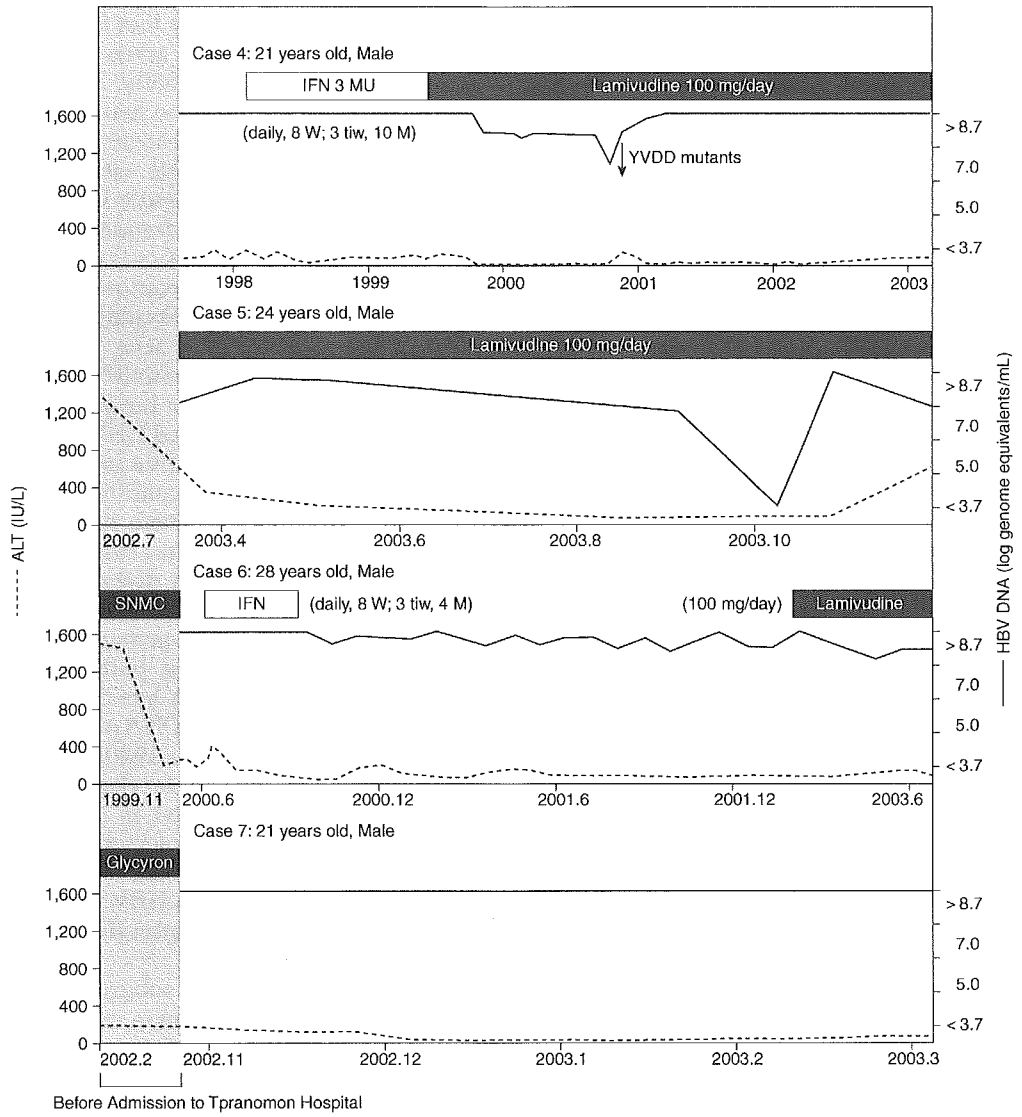

Fig. 2. Clinical courses of the four patients in whom HBeAg persisted after contracting infection with HBV genotype A despite antiviral treatment. The patient in Case 9 did not receive treatment after the admission. Courses and treatment they received before admission to Gastroenterology Department in Tornomon Hospital are shown in shaded areas on the **left**. IFN, interferon; SNMC, Stronger Neo-Minophagen C.

transfer to the Toranomon Hospital. Cases 4 and 6 did not respond to IFN or lamivudine that was commenced immediately after IFN or at an interval; lamivudine has been continued on them indefinitely. Case 5 was started on lamivudine soon after he was admitted to hospital and had been maintained on it for 1 year; he never responded to lamivudine. Variants with mutations in the YMDD motif of DNA polymerase/reverse-transcriptase developed in Case 4 while he was receiving lamivudine accompanied by a rise in ALT levels.

Although serum ALT returned to normal spontaneously (<50 IU/L) and then elevated only moderately in Case 7, high levels of HBV DNA (>8.7 LGE/ml) persisted through more than 1 year. Antiviral treatment was withheld because of the absence of active hepatitis.

DISCUSSION

There are marked geographical differences in the distribution of HBV genotypes [Magnius and Norder, 1995; Lindh et al., 1997; Miyakawa and Mizokami, 2003]. Of them, genotype A is not indigenous in Japan where genotypes B and C prevail and account for by far the majority of acute as well as chronic HBV infections [Orito et al., 2001; Kobayashi et al., 2002]. Some characteristics of HBV genotype A infection are increasingly coming to the fore in Japan and have aroused concerns in hepatologists at hospitals in urban areas with cosmopolitan populations. Ogawa et al. [2002] found that 14 of the 25 (56%) patients with acute hepatitis B in a downtown Tokyo (Shinjuku) were

infected with HBV genotype A. Moreover, the frequency of acute hepatitis induced by HBV genotype A in our hospital is higher after than before 1991 (2/22 [9%] vs. 26/46 [57%], $P < 0.0001$) [Kobayashi et al., 2004]. The present study sums up our experiences on acute infection with HBV genotype A at the Department of Gastroenterology in Toranomon Hospital situated in the Metropolitan Tokyo during the past 28 years, to supplement our previous reports with additional findings and new insights [Kobayashi et al., 2002, 2003, 2004].

First, infection with HBV genotype A spreads principally by extramarital sexual contact in the adulthood in Japan [Kobayashi et al., 2002; Ogawa et al., 2002]. All the 31 patients of acute hepatitis B infected with HBV genotype A in the present series were men, and 16 (52%) of them confided having had extramarital heterosexual or homosexual contacts. Only one mother of 32 patients with acute or chronic infection with HBV genotype A possessed HBV DNA in serum; her genotype was B [Kobayashi et al., 2003], thereby excluding perinatal transmission of genotype A. In a molecular epidemiological survey of HBV in Amsterdam, a cluster of genotype A related in men having sex with men has been recognized [van Steenbergen et al., 2002].

Secondly, acute infection with HBV genotype A tends to persist. Of the 31 patients with acute genotype A infection, seven (23%) failed to clear it within 6 months, in comparison with one of the nine (11%) with acute genotype B or three of the 42 (7%) with acute genotype C infection. In our previous report [Kobayashi et al., 2002], infection persisted in all three patients infected with genotype A, in contrasted to the clearance of HBsAg in all four with genotype B (one) or C (three).

Low maximum ATL levels (<500 IU/L [83%] vs. ≥500 IU/L [4%], $P = 0.0001$) and the high baseline HBV DNA levels (median: >8.7 vs. 6.0 LGE/ml, $P = 0.004$) were predictive of the perpetuation of acute HBV genotype A infection. Hence, compromised immune responses toward lower inflammation activity in the liver and higher viral replication may have a role in evolving HBV genotype A infection. Four of the seven (57%) patients who progressed to chronic were homosexuals. It is tempting to speculate that derangement in cytotocxic T cell response contributed to the failure in clearing acute HBV infection toward persistence [Handzel et al., 1984]. Immunomodulatory treatments to cope with severe acute hepatitis, given to five of the seven (71%) patients before referral to hospital (Figs. 1 and 2), may have promoted the persistence of infection with HBV genotype A. We have reported that acute prolonged HBV infection occurs more often in patients with than without immunomodulatory treatments during acute illness, regardless of genotypes (86% [6/7] vs. 2.4% [1/42], $P = 0.01$).

Thirdly, HBV genotype A infection persisting in patients with acute hepatitis B is not cleared often by antiviral therapy. HBV genotype A infection was terminated in only one of the six (17%) patients who received antiviral treatment. He was one of the three patients who seroconverted with the loss of HBeAg; interferon (IFN) and lamivudine was given to him early in the course of infection (Cases 1 in Fig. 1). Since most (~95%) patients with acute adulthood hepatitis B resolve infection in Japan, antiviral treatment is rarely used for them. As far as acute infection with HBV genotype A is concerned, however, therapeutic intervention needs to be considered in view of the frequent chronic outcomes. Since many (76% [24/31]) patients even with HBV genotype A can clear infection spontaneously, the timing of starting antiviral therapy would have to be contemplated. The single patient who cleared HBsAg was started on IFN and then lamivudine within 3 months after he was referred to our hospital. It is not certain whether he could have cleared HBV infection, should he never be placed on lamivudine early. HBsAg was not cleared, however, in the remaining two patients with HBeAg seroconversion in whom IFN was started 4 and 2 years, respectively, after they came to our care (Fig. 1).

Early antiviral treatment deserves consideration in patients who are infected with HBV genotype A, especially because of its propensity to become chronic. It is not certain how long patients should receive lamivudine after HBV DNA has disappeared from the circulation. Inasmuch as cccDNA continues to be present in the liver [Brechot et al., 1980; Yotsuyanagi et al., 1998], even after HBsAg is cleared from serum, a therapeutic option would be to continue lamivudine until anti-HBs is detected in serum as in Case 1. In view of the poor immune responses with low ALT levels, which might be inherent to HBV genotype A infection among homosexual, such a special care would have to be taken for its treatment.

There are two genetic subgroups of genotype A designated Ae which is common in Europe (the original genotype A) and Aa which is frequent in Africa as well as Asia [Sugauchi et al., 2003]; Aa is equivalent to subgroup A′ described by Bowyer et al. [1997]. It strikes as a surprise that of the 68 patients who were infected acutely or chronically with HBV genotype A and admitted to the Toranomon Hospital, 54 (79%) possessed HBV of subgroup Ae (European type); HBV of subgroup Aa (African/Asian type) was found in only four (6%) [Kobayashi et al., 2004]; they all were infected persistently. Since subgroup Ae was not found in any patients with acute hepatitis B in our series, it remains unclear whether or not the outcome of primary infection with HBV genotype A would be influenced by subgroup Aa and Ae.

Although acute HBV infection of genotype A tends to persist in comparison with those of the other genotypes, only a minority (7/31 [23%]) develops chronic infection. An efficient therapeutic strategy has to be found, however, since the infection with HBV genotype A was terminated in only one of the six (17%) patients who were treated. Recently, adefovir dipivoxil was found to be effective for the treatment of chronic hepatitis B [Marcellin et al., 2003], and it may offer a reasonable option for resolving persistent HBV genotype A infection.

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HEPATOLOGY

Virological differences between patients infected with subtypes Ba and Bj of hepatitis B virus genotype B

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Abstract

Background: Hepatitis B virus (HBV) genotype B is classified into subtype Ba with the recombination with genotype C in the precore region plus core gene and subtype Bj without recombination. Virological and clinical differences between infections with subtypes Ba and Bj, however, are yet to be determined.

Methods: During 1976 through 2001, 224 patients visited Toranomon Hospital in Tokyo, Japan who were infected with HBV genotype B. Subtypes of genotype B were determined by sequencing HBV-DNA recovered from sera for detecting recombination with genotype C.

Results: Subtype Ba was detected in 53 patients (24%) and Bj in 167 (75%); subtypes were not able to be determined in the remaining four (1%). The only virological difference was that detection of hepatitis B e antigen at the presentation was more frequent in the patients infected with subtype Ba than those with Bj (63% vs 33%, $P = 0.016$). There were no differences in the distribution of liver disease of various forms between the patients infected with subtypes Ba and Bj at presentation. No differences were noted, either, in the development of liver cirrhosis or hepatocellular carcinoma, or the loss of hepatitis B surface antigen from serum, between the patients infected with subtypes Ba and Bj during follow up of up to 26 years.

Conclusions: Although there were some virological differences between the patients infected with subtypes Ba and Bj of HBV genotype B, they do not seem to influence the long-term clinical outcome.

Key words: hepatitis B e antigen, hepatitis B surface antigen, hepatitis B virus, genotypes, subtypes.

INTRODUCTION

Hepatitis B virus (HBV) is classified into seven genotypes designated by the letters from A to G.[1–3] Recently, an eighth genotype, named H, has been proposed that is closely related to genotype F.[4] Genotypes of HBV have distinct geographic distribution and they influence the clinical course of hepatitis B. Because genotypes A and D frequently occur in Western countries, while genotypes B and C are common in Asia, clinical differences between genotypes A and D, as well as B and C, have been studied extensively.

It has been reported that genotype A induces chronic liver disease more frequently[5] and is associated with better response to interferon than genotype D.[6] Another recent study, however, has found that sustained biochemical remission and clearance of HBV-DNA, as well as the clearance of hepatitis B surface antigen (HBsAg), occurred at a higher rate in genotype A- than in genotype D-infected patients.[7] There have been increasing lines of evidence for more severe liver disease and longer duration of hepatitis B e antigen (HBeAg) in serum, accompanied by delayed seroconversion to antibody to HBeAg (anti-HBe), in infection with HBV genotype C than B.[8,9] Furthermore, hepatocellular carcinoma (HCC) develops more frequently in the patients infected with HBV genotype C than B.[10] Clinical courses may differ, however, even among the patients

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