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Efficacy of lamivudine therapy in elderly patients with chronic hepatitis B infection

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Background. The aim of this study was to evaluate the efficacy of lamivudine therapy in elderly patients with chronic HBV infection. ***Methods.*** Patients aged ≥60 years (n = 40) received lamivudine monotherapy between February 1995 and September 2005 at Toranomon Hospital. We compared the efficacy of lamivudine therapy in these patients and in 639 patients aged <60 years, including 80 patients aged <60 years matched for sex, hepatitis B e antigen (HBeAg) status, and hepatitis B virus (HBV) DNA level. ***Results.*** The rates of normalization of alanine aminotransferase (ALT) level in 40 patients aged ≥60 years and 639 patients aged <60 years were 85% versus 76%, and 86% versus 73% at 1 and 3 years, respectively. The respective rates of loss of HBV-DNA were 74% versus 74%, and 76% versus 68% at 1 and 3 years. The respective cumulative emergence rates of the YMDD mutant were 16% and 17% at 1 year, and 46% and 49% at 3 years. In 80 patients <60 years old matched for sex, HBeAg status, and HBV-DNA level, the rates of normalization of the ALT level and loss of HBV-DNA were similar to those in the 639 patients aged <60 years. The emergence rate of YMDD mutants in patients aged ≥60 years were similar to those in matched patients aged <60 years. Multivariate analyses identified low serum bilirubin (<1 mg/dl) as an independent factor associated with the emergence of the YMDD motif mutation in patients aged ≥60 years. ***Conclusions.*** Our results suggest that treatment with lamivudine is both well tolerated and efficacious in elderly patients with chronic HBV infection.

Key words: HBV, elderly patients, lamivudine, YMDD mutant

Introduction

Chronic infection with hepatitis B virus (HBV) affects as many as 350 to 400 million people worldwide and 1.5 million people in Japan.[1] Vaccination is mainly used in Japan to prevent HBV infection via mother-to-infant transmission and to reduce the number of HBV carriers. However, there are still many patients with HBV infection. Moreover, elderly patients with chronic hepatitis are on the increase, and the potential for development of cirrhosis or hepatocellular carcinoma in such patients is real. Hence, treatment of elderly patients with HBV is an important issue.

Lamivudine is an oral cytosine nucleoside analog that potently inhibits HBV replication by interfering with HBV reverse transcriptase activity.[2–5] 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 e antigen (HBeAg).[3,5–12] 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.[13–15] We already reported the efficacy of lamivudine therapy and factors associated with the emergence of resistance in chronic HBV infection in Japan.[15] However, to our knowledge, there are no reports that describe the efficacy of lamivudine treatment in elderly patients (≥60 years) with chronic hepatitis B.

The aims of the present study were (1) to assess the benefits of lamivudine therapy for elderly patients (≥60 years) with chronic hepatitis B, and (2) to determine differences in the emergence rate of YMDD mutants and the appearance of breakthrough hepatitis between patients <60 years old and those ≥60 years old.

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Table 1. Characteristics of unmatched patients at commencement of lamivudine therapy

	Patients aged <60 ($n = 639$)	Patients aged ≥60 ($n = 40$)	*P* value
Age (years)[a]	42 (23–58)	63 (60–76)	<0.001
Sex (male/female)	523/116	23/17	0.001
Liver cirrhosis (%)	103 (16.1%)	7 (17.5%)	NS
Median duration of treatment (months)[a]	25 (6–135)	44 (6–69)	NS
Platelet count ($\times 10^4/\mu l$)[a]	18.5 (4.1–47.3)	13.3 (5.2–25.8)	0.018
Aspartate aminotransferase (IU/l)[a]	75 (15–2718)	81 (27–1309)	NS
Alanine aminotransferase (IU/l)[a]	106 (12–2437)	123 (16–928)	NS
Serum bilirubin (mg/dl)[a]	0.8 (0.3–20.2)	0.8 (0.3–3.6)	NS
Serum albumin (g/dl)[a]	3.9 (1.0–4.5)	3.6 (1.8–4.4)	0.01
HBV-DNA (LGE/ml)[a]	7.2 (<3.7–8.7)	6.8 (<3.7–8.7)	NS
HBeAg-positive (%)	376 (58.8%)	15 (37.5%)	0.01
HBV genotype (A/B/C/other)	20/38/536/45	0/4/32/4	NS

NS, not significant; HBV, hepatitis B virus; LGE, $\log_{10}$ genome equivalents; HBeAg, hepatitis B e antigen
[a] Values are median (range)

Table 2. Characteristics of patients matched for sex, HBeAg status, and HBV-DNA level at commencement of lamivudine therapy

	Patients aged <60 ($n = 80$)	Patients aged ≥60 ($n = 40$)	*P* value
Age (years)[a]	44 (23–58)	63 (60–76)	<0.001
Sex (male/female)	46/34	23/17	Matched
Liver cirrhosis (%)	13 (16.0%)	7 (17.5%)	NS
Median duration of treatment (months)[a]	44 (6–118)	44 (6–69)	NS
Platelet count ($\times 10^4/\mu l$)[a]	16.5 (4.1–47.3)	13.3 (5.2–25.8)	0.017
Aspartate aminotransferase (IU/l)[a]	61 (21–1656)	81 (27–1309)	NS
Alanine aminotransferase (IU/l)[a]	80 (12–1854)	123 (16–928)	NS
Serum bilirubin (mg/dl)[a]	0.7 (0.3–12.2)	0.8 (0.3–3.6)	NS
Serum albumin (g/dl)[a]	3.8 (1.0–4.5)	3.6 (1.8–4.4)	0.05
HBV-DNA (LGE/ml)[a]	6.7 (<3.7–8.7)	6.8 (<3.7–8.7)	Matched
HBeAg positive (%)	30 (37.5%)	15 (37.5%)	Matched
HBV genotype (A/B/C/other)	2/9/66/3	0/4/32/4	NS

[a] Values are median (range)

Patients and methods

Patients

Between February 1995 and September 2005, 40 consecutive Japanese patients aged ≥60 years were enrolled in this study at Toranomon hospital, Tokyo. All patients fulfilled the following criteria: (1) presence of hepatitis B surface antigen (HBsAg) in serum (positive for HBsAg for >6 months); (2) HBV-DNA positivity by quantitative assay; (3) absence of hepatoma; (4) absence of coinfection with hepatitis C virus (HCV); and (5) no previous treatment with any nucleoside analog.

The baseline characteristics of the 40 patients included in the study are listed in Table 1. All patients were Japanese; 23 were men and 17 were women; 15 were HBeAg-positive and 25 were HBeAg-negative; 32 patients had genotype C, four had genotype B, and the genotype was unknown in four. To determine the efficacy of lamivudine therapy and emergence rate of YMDD mutants, we compared the 40 patients aged ≥60 years with another group of 639 patients aged <60 years with HBV-related chronic infection on lamivudine therapy in our hospital. The baseline characteristics of the 679 patients are also listed in Table 1.

Since sex and HBeAg status were significantly different between the two age groups, we selected 80 patients from the 679 patients aged <60 years with HBV-related chronic infection on lamivudine therapy in our hospital who were matched to the ≥60 age group with respect to sex, HBV-DNA level, and HBeAg status (Table 2). The median duration of lamivudine therapy in the 40 patients aged ≥60 years was 44 months (range, 6–69 months). All comparisons described in this study pertain to two age groups of patients matched for sex, HBeAg status, and HBV-DNA levels unless otherwise stated.

One patient requested termination of lamivudine therapy and died from hepatocellular carcinoma during the observation period. He received lamivudine therapy for 16 months. Baseline platelet count and serum albumin concentrations differed between the ≥60-year-old and <60-year-old groups. Although the numbers of patients with genotypes A and B were small, the distribution of the HBV genotype was similar in patients with chronic HBV infection who had received care in our hospital over a follow-up period of more than 2 years.[16] Other factors were not significantly different between the two age groups.

Methods

All patients were treated with lamivudine at a dose of 100 mg/day orally, given continuously for at least 6 months, after providing informed consent. Clinical and laboratory assessments were performed once a month. Chronic hepatitis or cirrhosis was confirmed by needle biopsy, peritoneoscopy, or clinical criteria before treatment. The clinical criteria for chronic hepatitis included elevated alanine aminotransferase (ALT) levels over 6 months; absence of clinical evidence of portal hypertension, such as esophageal varices, ascites, or hepatic encephalopathy; and imaging features suggestive of cirrhosis on ultrasonography.[15] The diagnosis of chronic hepatitis and cirrhosis was established in 33 and 7 patients, respectively. HBeAg loss was defined as undetectable HBeAg. After the emergence of the YMDD mutant, we added adefovir dipivoxil (ADV) to ongoing lamivudine therapy. Three (5%) patients aged >60 years were treated with ADV in addition to lamivudine. On the other hand, 17 (28.3%) patients aged <60 years were treated with ADV in addition to lamivudine.

Laboratory and virological tests

Routine biochemical tests were performed at least once a month, before and during therapy, using standard procedures. 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). Mutations in the YMDD motif in the polymerase gene were determined using polymerase chain reaction and restriction fragment length polymorphism, by a method described previously.[14] Lamivudine resistance was determined annually before the development of mutations, and, if a mutation appeared, the time of appearance of resistance was confirmed by monthly measurement.

Data analysis

Kaplan-Meier analysis and the log-rank test were used to estimate and compare the rates of viral resistance and appearance of breakthrough hepatitis between the ≥60-year-old and <60-year-old groups. A two-tailed P value of less than 0.05 was considered statistically significant. Differences between groups were examined for statistical significance using the Mann-Whitney U test and χ-squared test where appropriate. Independent risk factors associated with emergence of YMDD motif mutation were studied using stepwise Cox regression analysis. Potential risk factors for emergence of the YMDD motif mutation that were assessed included the following ten variables: sex, degree of liver disease (cirrhosis or not), platelet count, aspartate aminotransferase (AST), ALT, serum bilirubin, serum albumin, HBV-DNA level, HBeAg, and HBV genotype. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. All factors found to be at least marginally associated with emergence of mutation of the YMDD motif ($P < 0.15$) were entered into the multivariate Cox proportional hazard model. A P value less than 0.05 was considered statistically significant. All analyses described above were performed using the SPSS program (version 7.5, SPSS, Chicago, IL, USA).

Results

Serum HBV-DNA and ALT concentrations

The rates of normalization of ALT level following lamivudine treatment for the 40 patients aged ≥60 years and the 639 patients aged <60 years were 85% versus 76%, 89% versus 75%, and 86% versus 73% at 1, 2, and 3 years, respectively. Furthermore, the respective rates of loss of HBV-DNA were 74% versus 74%, and 68% versus 71%, and 76% versus 68% at 1, 2, and 3 years, respectively. The cumulative emergence rates of YMDD mutant in patients aged ≥60 years and those <60 years were 16% and 17% at 1 year, 32% and 36% at 2 years, and 46% and 49% at 3 years. The rates of normalization of ALT level, loss of HBV-DNA, and emergence of YMDD mutants in patients aged ≥60 years were similar to those in the younger age group.

Figures 1 and 2 show the rates of normalization of ALT level and nondetection of HBV-DNA at 6 months and 1, 2, and 3 years in the patients matched for sex, HBeAg status, and HBV-DNA level during lamivudine therapy. The ALT normalization rate of the <60-year-old group tended to decrease year by year. On the other hand, the rate of the ≥60-year-old group tended to be higher than that of <60-year-old group at 2 and 3 years, although the difference was not significant (Fig. 1).

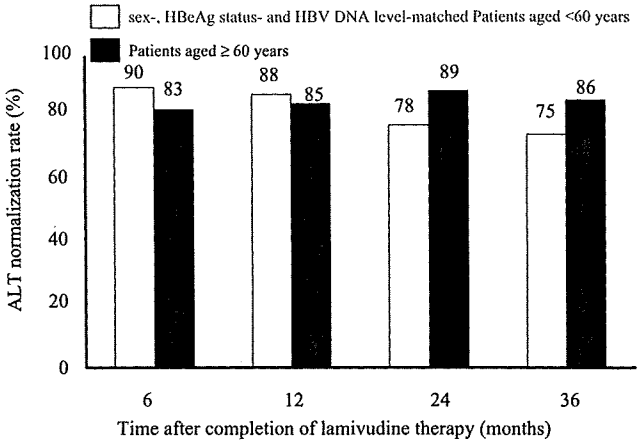

Fig. 1. Alanine aminotransferase (*ALT*) normalization rate. *Numbers above the bars* represent the values of normalization rates in patients of the two age groups matched for sex, hepatitis B e antigen (*HBeAg*) status, and hepatitis B virus (*HBV*)-DNA level

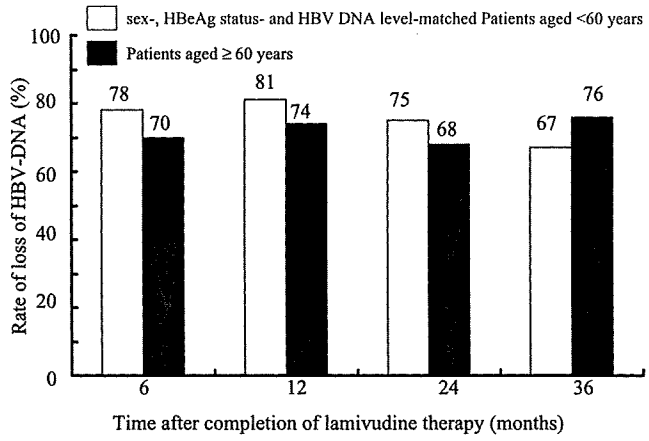

Fig. 2. HBV-DNA loss rate. *Numbers above the bars* represent the values of loss rates in patients of the two age groups matched for sex, HBeAg status, and HBV-DNA level

Figure 2 shows HBV-DNA loss rates in both groups. In patients aged <60 years, HBV-DNA and ALT levels at 2 and 3 years were lower than those at 6 months and 1 year, reflecting the development of viral resistance. In patients aged ≥60 years, the HBV-DNA loss rate was almost stable at all time points. HBeAg seronegative rates were 36% versus 37% at 1 year, 46% versus 41% at 2 years, and 46% versus 55% at 3 years for patients aged ≥60 years and <60 years, respectively (differences not significant).

Emergence of YMDD motif mutant and appearance of breakthrough hepatitis

The cumulative emergence rates of the YMDD mutant in patients aged ≥60 years and <60 years were 16% and

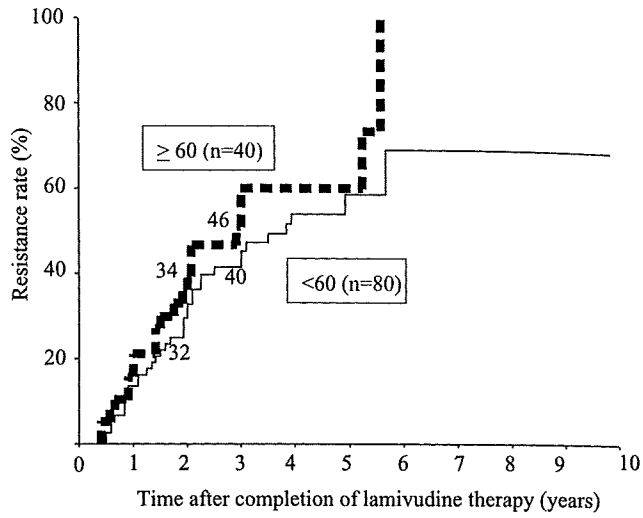

Fig. 3. Cumulative percentages of sex-, HBeAg status-, and HBV-DNA level-matched patients (<60 and ≥60) who showed viral resistance during treatment with lamivudine (Kaplan-Meier analysis). *Numbers* represent the actual percentages for the indicated intervals

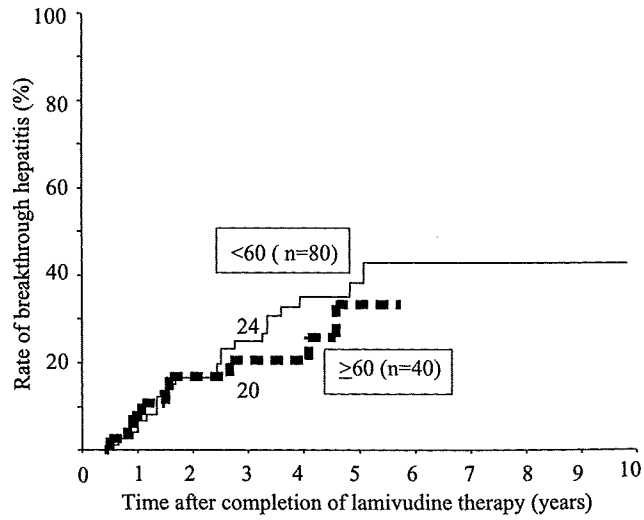

Fig. 4. Cumulative percentages of sex-, HBeAg status-, and HBV-DNA level-matched patients (<60 and ≥60) who showed breakthrough hepatitis during treatment with lamivudine (Kaplan-Meier analysis). *Numbers* represent the actual percentages for the indicated intervals

17% at 1 year, 32% and 34% at 2 years, and 46% and 40% at 3 years, respectively (Fig. 3). The emergence rates of YMDD mutants in patients aged ≥60 years were similar to those in patients aged <60 years at all three time intervals. The cumulative breakthrough hepatitis appearance rates in patients aged ≥60 and <60 years were 14.0% and 12.0% at 1 year, 12.0% and 12.0% at 2 years, and 20% and 26.0% at 3 years, respectively (not significant, Fig. 4). However, the appearance rate of

Table 3. Laboratory data for patients matched for sex, HBeAg status, and HBV-DNA level after emergence of YMDD mutant during lamivudine therapy

	<60 (n = 36)	≥60 (n = 21)	P value
Maximum ALT (IU/l)[a]	69 (15–727)	24 (12–1669)	0.049
Maximum bilirubin (mg/dl)[a]	0.9 (0.3–3.4)	0.7 (0.3–1.2)	NS
Maximum HBV-DNA (LGE/ml)[a]	6.5 (2.6–8.7)	4.6 (2.6–8.7)	NS

ALT, alanine aminotransferase
[a] Values are median (range)

breakthrough hepatitis in patients aged ≥60 years tended to be lower than in those <60 years, although the difference was not statistically significant.

These characteristics of patients with emergence of the YMDD mutant (sex, type of YMDD mutant, cirrhosis, duration of therapy, HBeAg, HBV genotype and HBV-DNA) were not different between the ≥60-year-old and the <60-year old groups. Table 3 summarizes the laboratory data of patients after emergence of the YMDD mutant. The maximum ALT in patients aged ≥60 years was significantly lower than that in patients aged <60 years (P = 0.049).

Twenty-two (55%) patients among patients aged ≥60 years developed mutations in the YMDD motif during lamivudine therapy. We then explored the risk factors for the emergence of the YMDD motif mutation in patients aged ≥60 years. In univariate analyses, the following three factors significantly influenced the emergence of resistance: low AST level (P = 0.045), low ALT level (P = 0.028), and low bilirubin levels (P = 0.001). Since the variables were mutually correlated, multivariate analysis was performed. That analysis identified a low bilirubin level (hazard ratio, 14.40; 95% confidence interval, 2.89–71.82; P = 0.001) to be a significant determinant of emergence of the YMDD motif mutation. On the other hand, in patients aged <60 years, we identified high HBV-DNA level as a significant determinant of emergence of the YMDD motif mutation.

Discussion

The benefits of lamivudine in patients with compensated HBV-related liver disease have been suggested by several groups.[3–7] Our study is the first to show long-term efficacy by lamivudine monotherapy in older patients. Our study demonstrated that lamivudine therapy was well tolerated by elderly Japanese patients and led to reductions in levels of transaminases and HBV-DNA prior to the emergence of YMDD mutants.

The emergence of YMDD mutants is known to be associated with high HBV-DNA levels, high ALT levels, and HBeAg-positivity at baseline, especially among patients with chronic hepatitis.[15,17–21] In our study, sex and HBeAg status were significantly different between the ≥60-year-old and <60-year-old groups. Therefore, we selected 80 patients from 639 patients aged <60 years with HBV-related chronic hepatitis on lamivudine therapy in our hospital, matched with respect to sex, HBV-DNA level, and HBeAg status.

In patients aged <60 years, the normalization rate of transaminases tended to decrease year by year; for example, the rates were 78% at 2 years and 75% at 3 years, because breakthrough hepatitis appeared in some patients. However, compared with patients aged <60 years, the high normalization rate of transaminases was sustained during follow-up in patients aged ≥60 years. Moreover, HBV-DNA loss rates were sustained during follow-up in patients aged ≥60 years.

In elderly patients with HBV, we identified low AST levels, low ALT levels, and low bilirubin levels by univariate analysis as associated factors. These results are similar to those reported previously by others.[19,22] Moreover, multivariate analysis identified low bilirubin levels as a significant predictor. In comparison, a high HBV-DNA level was not a predictive factor in patients aged ≥60 years, although in patients aged <60 years, high HBV-DNA levels were identified as a predictive factor (P = 0.029). That high HBV-DNA level was not a predictive factor in patients aged ≥60 years may be due to the small number of patients.

The emergence rate of YMDD mutants in patients aged ≥60 years was similar to that in patients aged <60 years. On the other hand, the appearance rate of breakthrough hepatitis tended to be lower in patients aged ≥60 years than in those <60 years of age, although the difference was not statistically significant. The reason for the low frequency of breakthrough hepatitis in patients aged ≥60 years in spite of the same emergence rate of the YMDD mutant remains uncertain. The characteristics of patients with emergence of the YMDD mutant were not significantly different between the two age groups. Moreover, the maximum ALT in patients aged ≥60 years tended to be lower than that in patients aged <60 years. Nevertheless, one explanation may be that the immune response was lower in the elderly,[23] probably owing to a decrease in T helper cell function and in the responsiveness of B cells with age.[24,25] Further virological and/or immunological studies are necessary to investigate this issue.

Taken together, our results suggest that lamivudine therapy is safe in elderly persons. However, some of these patients with HBV could not receive interferon therapy because of the risk of potentially life-threatening complications. Therefore, the indications for therapy in elderly patients with breakthrough hepatitis must be carefully considered. These patients are in need of other antiviral agents with anti-HBV activity. Recent studies suggest that adefovir dipivoxil and entecavir may effectively suppress YMDD mutants.[20,21,26,27] However, the efficacy and safety of these agents in the elderly with HBV with YMDD mutants have not yet been established. A combination therapy with lamivudine and other anti-HBV agents may induce a decrease in the frequency of drug resistance and delay progression in elderly patients with HBV.

In conclusion, our results suggested that lamivudine therapy improved the clinical course in elderly patients. Lamivudine therapy for elderly Japanese patients was well tolerated and resulted in reduction of transaminases and HBV-DNA levels. The rate of breakthrough hepatitis in patients aged ≥60 years was lower than that in patients aged <60 years in spite of the frequent emergence rate of the YMDD mutant. Moreover, breakthrough hepatitis tended to occur in patients aged <60 years more than in patients aged ≥60 years. Further studies are needed to evaluate, under careful virological monitoring, whether new antiviral agents such as adefovir dipivoxil and entecavir are useful in elderly patients with HBV.

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Case report

Selection of a virus strain resistant to entecavir in a nucleoside-naive patient with hepatitis B of genotype H

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Keywords: Hepatitis B virus; Entecavir; Genotype H

1. Introduction

Recently, entecavir has provided significantly better rates of virologic and biochemical improvement than lamivudine in patients with chronic hepatitis B (CH-B) not previously receiving a nucleoside analogue (Chang et al., 2006; DeMan et al., 2001; Lai et al., 2002). Rare evidence of emerging variants to entecavir among these patients has emerged (Colonno et al., 2006). On the other hand, HBV has been classified into genotypes A through H in the full genome sequence (Norder et al., 2004). Under this classification, genotype H has been recently identified in Nicaragua, the USA and Mexico (Araus-Ruiz et al., 2002). A further three reports of nucleotide sequence cases of genotype H HBV have been reported in Japanese patients (Nakajima et al., 2005; Ohnuma et al., 2005; Shibayama et al., 2005). Nevertheless, genotype H is extremely rare and the efficacy of nucleoside analogue treatment against it has not been determined. To our knowledge, this is the first report of emerging resistance to entecavir in a nucleotide-naive patient with genotype H, the rarest genotype internationally.

2. Case report

A 38-year-old Japanese man with CH-B received a check-up in November 2001, and was found to be seropositive for hepatitis B surface antigen with mild alanine aminotransferase (ALT) elevation. He was diagnosed with CH-B by peritoneoscopy and liver biopsy (moderate hepatitis [A2] and severe fibrosis [F3]) at another hospital in June 2002. HBeAg was positive and serum HBV DNA was 7.6 log copies/mL (Roche Amplicor HBV Monitor assay, Roche Diagnostics, Indianapolis, IN). He was admitted to our hospital in July 2002. At this time, treatment with interferon α was started, but his ALT level remained elevated and HBeAg was positive at 24 weeks of administration. He was enrolled in a phase II randomized (1:1:1:1) trial of entecavir and lamivudine by repeat oral administration of entecavir 0.01 mg, 0.1 mg, or 0.5 mg or lamivudine 100 mg for 24 weeks. Treatment with entecavir was started at 0.1 mg/day in April 2004, at which time serum HBV DNA was >7.6 log copies/mL and ALT was 100 IU/L. At 24 weeks, he was switched from entecavir 0.1 mg directly to 0.5 mg without any break in administration. After the start of entecavir, ALT levels increased temporarily and then decreased to within normal values by January 2007. At 44 weeks of entecavir, serum HBV DNA was less than 4 log copies/mL and remained around this level until September 2006 (124 weeks), when it again began to increase (Fig. 1).

Full genome sequence analysis before treatment with entecavir revealed that the patient was infected with genotype H virus (Fig. 2). The sequence was named HBV-ST0404. When compared with previously reported HBV isolates with full genome sequences, ST0404 showed high overall identity (99.0%) with a prototype of the Los Angeles strain (AY090460) and 97.5% identity with a Nicaragua strain

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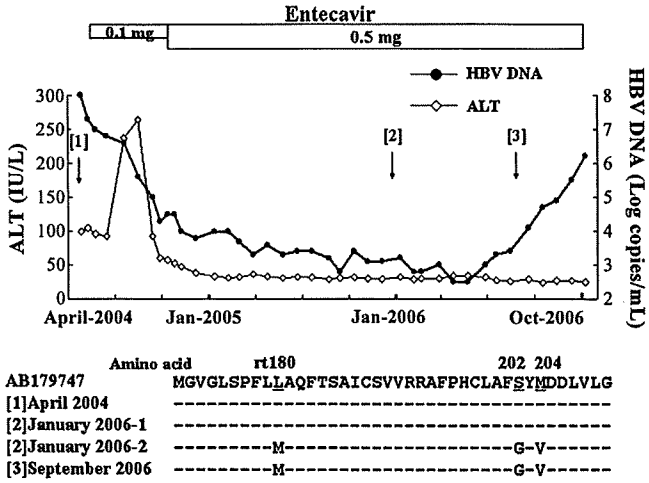

Fig. 1. Clinical course and evolution of viral polymerase reverse transcriptase gene sequence during the entecavir therapy. AB179747 was a strain reported by Ohnuma et al. (2005). In January 2006, there were two kinds of strains (January 2006-1 and -2).

(AY090457) of the genotype H group at the nucleotide level. Moreover, ST0404 showed higher overall identity (99.6%, 99.4% and 98.8%) with Japanese strains (AB179747, AB205010 and AP007261, respectively) (Nakajima et al., 2005; Ohnuma et al., 2005; Shibayama et al., 2005). The sequence of the viral polymerase reverse transcriptase (rt) gene at the baseline of entecavir therapy was wild-type (April 2004). Moreover, YMDD mutants were not detected at baseline of entecavir therapy by PCR with peptide nucleic acid clamping (PNA) method (Matsuda et al., 2004). Nucleotide sequences of the polymerase gene during treatment were additionally determined by direct sequencing (Suzuki et al., 2006), with sequences of 10–30 independent clones for samples at a number of points determined and analyzed. In January 2006 (88 weeks), amino acid substitutions of rt gene, rtL180M, rtM204V and rtS202G, were simultaneously detected. From January 2006 to July 2006, the sequencing of independent clones clarified that these substitutions co-existed with wild-type. Since September 2006, these substitutions have been present as the major circulating variants and were associated with an increase in serum HBV DNA (Fig. 1).

3. Discussion

In two randomized double-blind trials of entecavir administered orally at 0.5 mg once daily for 52 weeks to HBeAg-positive, or HBeAg-negative nucleotide-naive patients, entecavir was superior to lamivudine in the primary efficacy endpoints of reduction of viral load and normalization of ALT levels (Chang et al., 2006; Lai et al., 2006). Moreover, no evidence of emerging resistance to entecavir was seen by 48 weeks in either study.

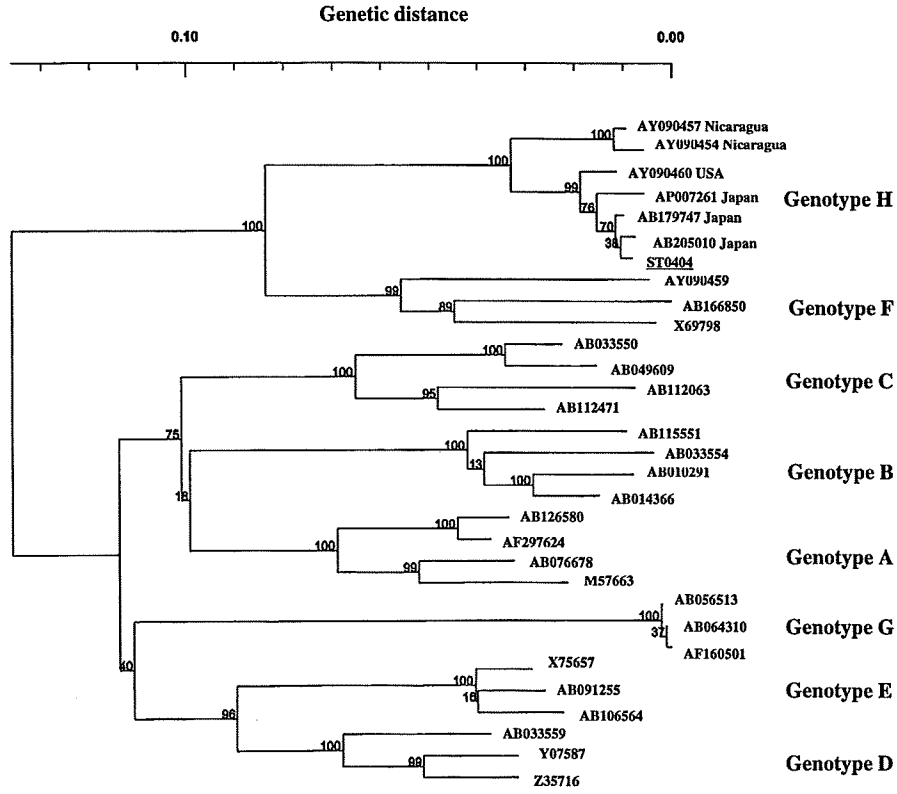

Fig. 2. Phylogram generated by neighbor-joining analysis of genetic distance in the full-length sequence of HBV. Thirty strains (without ST0404; indicated in underline) were retrieved from the GenBank/EMBL/DDBJ database.

However, recently report showed entecavir resistance emergence in entecavir-treated nucleotide-naive patients over a 2-year period (Colonno et al., 2006). The rate of emerging entecavir resistance was rare (0.1% and 0.3% at 1 year and 96 weeks treated, respectively).

In the present study, we describe the selection of rtL180M, rtS202G and rtM204V, which were associated with entecavir resistance (Tenney et al., 2004; Yim et al., 2006), in a nucleotide-naive patient. These entecavir resistance-associated substitutions were detected by the direct sequence and cloning method at 88 weeks of entecavir treatment. Interestingly, these substitutions emerged simultaneously. In past investigations of lamivudine-resistant viruses (rtL180M and rtM204V), additional substitutions at rtT184, rtS202, or rtM250 were shown to further reduce entecavir susceptibility (Tenney et al., 2004; Yim et al., 2006). Colonno et al. (2006) reported that one entecavir-treated nucleotide-naive patient, who had obvious lamivudine-resistance substitutions at baseline by sequencing, subsequently developed a S202G substitution and proceeded to have a virologic rebound during treatment with 0.5 mg entecavir. Moreover, another entecavir-treated nucleotide-naive patient did not have a virologic rebound but had genetically linked lamivudine and entecavir resistance-associated substitutions appear simultaneously. In our case, there were no YMDD mutants at baseline of entecavir therapy by PCR-PNA method. We could detect 0.01% of mutant viruses co-existing in 10^9 copies of wild-type viruses using this highly sensitive assay (Matsuda et al., 2004). Therefore, our case was same pattern of the latter and it may be difficult to predict emergence of entecavir resistance at baseline of entecavir therapy. On the other hand, HBV polymerase substitutions associated with adefovir dipivoxil were not related to lamivudine resistance. Taken together, these findings indicate that lamivudine and entecavir may exhibit selective pressure on similar subdomains of the viral rt, and that adefovir dipivoxil may put selective pressure on different subdomains of the rt lesion. Of particular clinical importance, entecavir monotherapy in nucleotide-naive patients may introduce the selection of entecavir-resistant strains (rtL180M, rtM204V and rtS202).

Recent reports have described HBV genotype H from strains derived from Nicaragua, Mexico and Los Angeles, and noted its close phylogenetic relationship to genotype F (Araus-Ruiz et al., 2002). In Japan, genotype H is extremely rare, with the Japan Red Cross NAT Screening Research Group recently reporting it in only 1 of 328 (0.3%) HBV DNA-positive Japanese blood donors (Ohnuma et al., 2005). Moreover, the strain in our case showed high homology with those reported in Japan (Nakajima et al., 2005; Ohnuma et al., 2005; Shibayama et al., 2005) and Los Angeles (Araus-Ruiz et al., 2002). In the Japanese patient reported by Nakajima et al. (2005), the infection was suggested to have occurred in Thailand. The genotype H HBV spreading in East Asia is therefore most closely related to the prototype of the Los Angeles strain.

To date, the relationship between the emergence of entecavir resistance and genotype was reported. In entecavir therapy in chronic hepatitis B patients who were refractory to lamivudine, entecavir resistance emerged in genotypes A to D (Tenney et al., 2004; Yim et al., 2006). Given that the sequences of domains B and C containing entecavir resistance (rtL180, rtT184, rtS202 and rtM204) are highly conserved between genotypes, it is feasible that entecavir-resistant virus should emerge in patients with genotype H. However, only a few reports have investigated resistance and the clinical and virological features of this condition are not clear. The present case of entecavir resistance is particularly notable for its emergence in a case of genotype H infection, which is rare, and in the likely absence of preexisting lamivudine resistance. Investigation of entecavir therapy in a large number of nucleo side-naive patients is warranted.

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Prolonged-Interferon Therapy Reduces Hepatocarcinogenesis in Aged-Patients With Chronic Hepatitis C

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The aim of this study was to elucidate the reduction of hepatocarcinogenesis by prolonged interferon (IFN) monotherapy in aged chronic hepatitis C patients. Inclusion criteria were biopsy-proven chronic hepatitis or liver cirrhosis, 60 years and over, elevated serum aminotransferase and positive hepatitis C virus (HCV)-RNA. One hundred and twenty patients satisfied the above criteria were treated with natural IFN-alpha (dose: 3 million unit (MU), two or three times weekly for 0.5–15.5 years, mean 2.47 years) (IFN group). Another 240 patients treated with herbal medicines excluding IFN were selected as control (no-IFN group). The patients not treated with IFN were matched 2:1 with IFN group patients for sex and age. The clinical and biological differences were compared after treatment with the IFN group and the untreated group. Serum alpha-fetoprotein (AFP) level decreased with statistical significance after initiation of treatment with IFN compared to no treatment. The 5- and 10-year cumulative rates of hepatocellular carcinoma (HCC) were 5.9 and 13.7%, and 17.1 and 32.8%, for the IFN and untreated group, respectively. HCC development occurred when histologic staging was advanced, and IFN was not given, the AFP level after treatment was >10 ng/ml. Cox regression analysis indicated that the relative risk of HCC in patients in the IFN group was 0.3 times of that in the untreated patients. The relative risk rate for HCC in severe fibrosis was 3.9 compared with mild or moderate fibrosis. In conclusion, long-term IFN therapy for aged patients with chronic HCV infection is effective in decreasing the serum AFP level and preventing hepatocarcinogenesis. ***J. Med. Virol. 79:1095–1102, 2007.***

KEY WORDS: chronic hepatitis C; hepatocellular carcinoma; long-term interferon treatment; alpha-fetoprotein

INTRODUCTION

Current interferon (IFN) therapy for patients with chronic hepatitis C viral infection has been directed at viral clearance. Recent studies described improvement of therapeutic efficacy when IFN was combined with ribavirin [Schalm et al., 1997; McHutchison et al., 1998; Poynard et al., 1998; Reichard et al., 1998]. Novel long-acting formulations of IFN known as pegylated IFN induced a higher eradication rate of hepatitis C virus (HCV) [Zuzem et al., 2000; Lindsay et al., 2001; Manns et al., 2001]. However, some patients do not clear the virus despite these new IFN therapies. Failure of HCV clearance could lead potentially to liver cirrhosis and/or hepatocellular carcinoma (HCC) [Imai et al., 1998; Yoshida et al., 1999]. Some patients cannot be given full doses of IFN because of IFN-related side effects. Thus, it is necessary to develop a new strategy for preventing the development of HCC in patients who cannot clear HCV-RNA regardless of IFN therapy and cannot be given full doses of IFN because of related side effects.

IFN can prevent the development of some malignancies apart from eradicating HCV [Gutterman, 1994;

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon.

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Fogler et al., 1994; Scarpa et al., 1997; Murphy et al., 2001; Lindner, 2002]. A few previous studies indicate that long-term IFN therapy reduces the development of HCC in patients with HCV and chronic hepatitis or cirrhosis [Nishiguchi et al., 1995; Ikeda et al., 2000]. However, there is also controversy as to whether patients should be treated to prevent the development of HCC.

Some patients in Japan with chronic hepatitis C were, generally, aged. Also, HCV-related HCC patients have been shown to become old with a peak around age 70. Thus, this match-controlled study was conducted to evaluate the effect of long-term IFN therapy on the development of HCC in aged patients with HCV and with chronic hepatitis or cirrhosis.

MATERIALS AND METHODS

Patients

The number of patients who were diagnosed with chronic HCV infection and were subsequently treated with IFN monotherapy or IFN and ribavirin combination therapy between April 1991 and March 2006 was 4,250. Seven hundred and twenty of these patients had the following criteria: (1) laparoscopy and liver biopsy which showed histopathological features of chronic hepatitis or cirrhosis was taken within 1 year of initiation of IFN therapy; (2) 60 years and over; (3) positive for HCV-RNA by the amplicor monitor assay [Albadalejo et al., 1998] or reverse transcription nested polymerase chain reaction (RT-nested PCR) [Hagiwara et al., 1992]; (4) average alanine aminotransferase (ALT) elevation greater than 1.5 times the upper normal limits (ALT normal range: 12–50 IU) for more than 6 months before IFN therapy; (5) no treatment with corticosteroids, immunosuppressive agents, or antiviral agents within 12 months; (6) negative for hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) in the serum, as determined by radioimmunoassay and spot hybridization; (7) Leukocytes $>2{,}500/mm^3$, platelets $>70{,}000/mm^3$, and bilirubin <2.0 mg/ml before the initial period of IFN therapy; and (8) no evidence of HCC nodules by ultrasonography and/or computerized tomography within 1 month before IFN therapy.

Of the 720 patients satisfied with above criteria, 120 received IFN to prevent the development of HCC at a dose of 3 million units (MU) of natural IFN-alpha (Sumitomo Pharmaceutical Co., Osaka, Japan) two or three times a week for 2.47 ± 2.65 years. The decision for IFN therapy was made mainly after discussion between physician and patient. The patients were prospectively monitored the serum aminotransferase, alpha-fetoprotein (AFP), and HCC development. On the other hands, out of 720 patients with the above criteria, 240 patients (no-IFN group) treated without IFN were selected retrospectively so that no-IFN group patients were matched 2:1 with IFN group patients for sex, ages, and severe fibrosis. Patients with either of the following criteria were excluded from the study: (1) AFP of 400 ng/ml or higher, (2) advanced and decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites, (3) a short follow-up period of 6 months or less, or (4) IFN was given daily, at a dose of >6 MU or other IFN excluded natural IFN-alpha. We compared the clinical and biological differences between IFN group and untreated group. The patients not treated with IFN were given herbal medicines (e.g., vitamin K, ursodeoxycholic acid, glycyrrhizin) [Takano et al., 1994; Arase et al., 1997; Tsubota et al., 1999]. Some of these substances improve serum transaminase and/or protecting HCC appearance [Takano et al., 1994; Arase et al., 1997; Tsubota et al., 1999]. Therefore, these drugs have been used for chronic hepatitis or cirrhosis in Japan since 1979. Untreated patient did not receive corticosteroids, immunosuppressive agents, or antiviral agents during the first stage of treatment. The study was approved by the institutional ethics review. Each patient gave informed consent.

Blood Tests

Blood samples were obtained just before treatment and stored at −80°C. HCV-RNA levels before therapy were treated by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems, CA) [Doglio et al., 1999]. Serum HCV-RNA every 2 or 3 month after the initiation of therapy in IFN group was examined by the qualitative PCR assay or RT nested PCR. The lower detection limit of the qualitative assay is 100 copies/ml. HCV genotype was examined by PCR, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously [Dusheiko et al., 1994].

Follow-Up Protocol

Follow-up began on the first day of IFN treatment. In control group, follow-up began on the first day of herbal medicines. Clinical evaluation and biochemical and hematological tests were undertaken at monthly intervals. Twenty-one patients were lost to follow-up. Because HCC did not develop in these 21 patients, they were removed from the subject of this study at the time of final consultation in statistical analysis [Harrington and Fleming, 1983]. Deaths unrelated to HCC and patients who started a new treatment in combination with corticosteroids, immunosuppressive agents, or antiviral drugs during the follow-up were also classified as withdrawals and removed from the study. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings on computed tomography and ultrasonography. Microscopic examination of fine-needle biopsy material was carried out in patients whose angiograms did not demonstrate a typical image of HCC.

Liver Histology

Liver biopsy specimens were obtained percutaneously or by peritoneoscopy using a modified Vim Silverman

needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas. Histopathological interpretations of specimens were made independently by experienced liver pathologists (YA and HK) who had no clinical information. 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 [Desmet et al., 1994]. Patients with severe fibrosis were considered as pre-cirrhosis or cirrhosis.

Statistical Analysis

Baseline characteristics and treatment differences of both groups were analyzed using Fisher's exact test or Krusukall Wallis test. HCC appearance rates were analyzed by the log rank test. A Cox proportional hazards model was used to analyze the factors contributing to the rate of development of HCC: factors examined included age, gender, histologic findings, HCV genotype, HCV load, aspartate aminotransferase (AST), ALT, and AFP. A P-value of <0.05 were considered statistically significant. The SPSS software package (SPSS 11.0 for windows; SPSS, Inc., Chicago, IL) was used for analyses.

RESULTS

Pretreatment Clinical Characteristics

Table I shows the characteristics of the patients with and without IFN treatment. There were no significant differences between the two groups with regard to sex ratio, age, histopathological stage of the liver, serum HCV-RNA level, and AST, ALT, AFP, and blood cell counts. In the control group, eighty-two patients started IFN during follow-up.

Changes in Serum AST, ALT, and AFP Activity After Treatment

Figure 1 shows the serum AST and ALT levels after initiation of treatment. The serum AST and ALT levels declined to normal levels after initiation of treatment with IFN. Transaminase levels at 6, 12, 18, and 24 months after in the IFN group were lower than that of patients not treated with IFN group with statistical significance. Figure 2 shows change in serum AFP level after initiation of treatment. Serum AFP level decreased after the initiation of IFN therapy compared the untreated group with statistical significance.

Loss of HCV-RNA

Of 120 patients treated with IFN, 18 patients lost serum HCV-RNA during IFN treatment. Of the 96 patients who stopped IFN therapy, 8 patients lost HCV-RNA in the serum 6 month after the termination of IFN treatment.

Cumulative Rates of HCC

HCC was diagnosed in four patients in the IFN group and 38 in patients no treated with IFN. Figure 3 shows the cumulative HCC development rates in both groups. The 5- and 10-year cumulative rates of HCC were 5.9 and 13.7%, and 17.1 and 32.8%, for IFN- and no-IFN groups, respectively. The cumulative rate of development of HCC in the IFN group was significantly lower than that not treated with IFN ($P = 0.045$).

Risk Factors for the Development of HCC

The rate of development of HCC after initiation of treatment, Cox regression analysis was performed using several variables. Univariate analysis showed that the following four factors affected significantly the cumulative development of HCC in all patients: histopathological staging ($P < 0.0001$), serum AFP level at 0.5 year after IFN therapy ($P = 0.005$), sex ($P = 0.006$), and IFN therapy ($P = 0.045$) (Table II). The variables were correlated mutually and multivariate Cox regression

TABLE I. Clinical Profiles Before Treatment

Characteristic	IFN group	Non-IFN group	P-value
N[a]	120	240	
Sex (M/F)	65/55	130/110	1
Age (years)[b]	63(60–75)	63(60–75)	1
Liver fibrosis (mild/moderate/severe)[a]	36/40/44	82/70/88	0.965
Genotype (1/2)[a]	85/31	170/61	0.782
HCV-RNA (KIU/ml)[b]	680(10–5,000)	720(5–5,000)	0.176
AST (IU/L)[b]	71(26–446)	67(20–355)	0.493
ALT (IU/L)[b]	86(38–699)	78(46–374)	0.101
AFP (ng/ml)[b]	10(3–316)	9(2–190)	0.342
Hemoglobin (g/dl)[b]	13.7(10.9–17.0)	14.0(11.8–17.0)	0.167
Platelet ($\times 104/mm^3$)[b]	12.8(5.2–25.6)	13.1(5.6–23.2)	0.275
WBC ($\times 103/mm^3$)[b]	4.0(2.1–11.3)	4.0(2.6–7.9)	0.570

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; WBC, white blood count; Normal reference ranges ≤ 10 ng/ml for AFP, 6–50 IU/L for ALT, 11–38 IU/L for AST.
[a]Data are number of patients.
[b]Data are median (range).

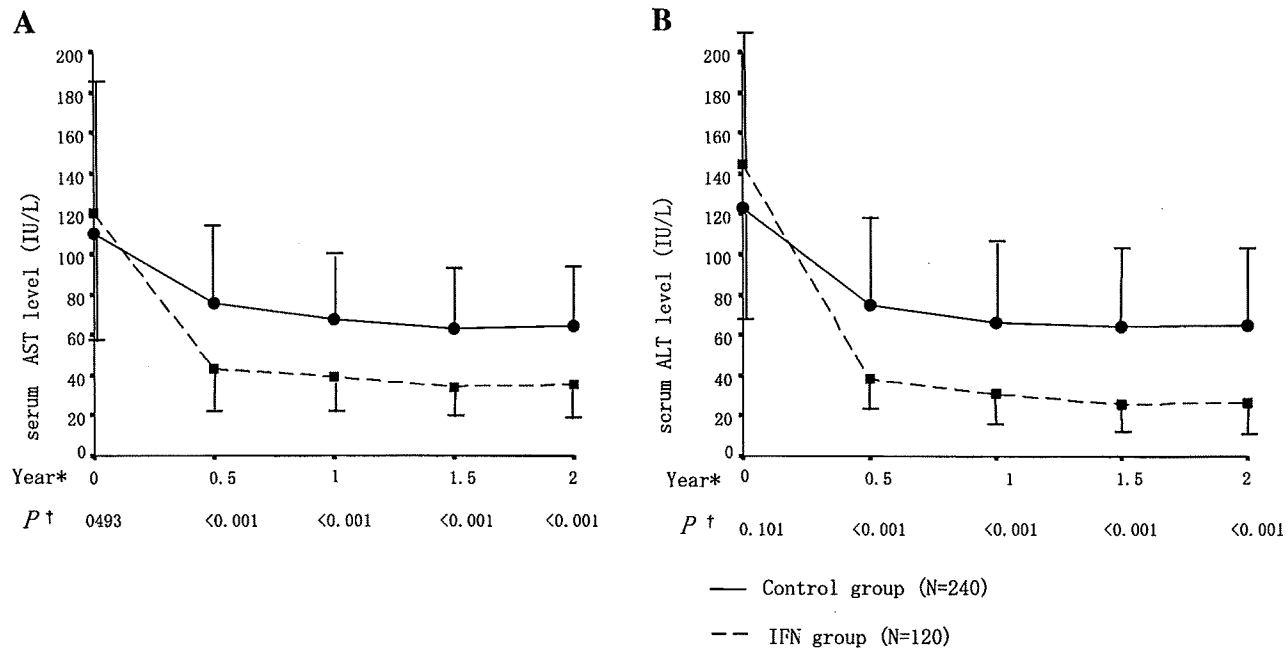

Fig. 1. Changes in serum AST (**panel A**) and ALT (**panel B**) after initiation of treatment. Data were expressed as mean ± standard deviation. *Year after initiation of treatment, †differences of both group by Krusukall Wallis test.

analysis was carried out with the four statistically significant variables in the model (Table III). The development of HCC occurred significant by when: (1) histological staging was advanced, (2) serum AFP level after the initiation of treatment was >10 ng/ml, and (3) IFN was not given. The relative risk of HCC in patients of the IFN group was 0.3 times of that in patients of no-IFN-group (Fig. 3). The relative risk for the development of HCC in patients with severe fibrosis was 3.9 compared to patients with mild or moderate fibrosis. Figure 4 shows the rate of development of HCC based on the difference of treatment and histological staging. IFN therapy could reduce significantly the development of HCC in severe fibrosis (Figure 4, Panel B).

Safety and Tolerance of IFN

Of the 120 patients included in this study, 9 discontinued IFN therapy because of adverse events: 3 cases of general fatigue, 2 cases of psychiatric disorder, 2 cases of aggravation of diabetes mellitus, 1 patient each with thrombocytopenia, pneumonia, and Parkinson's syndrome. The cumulative dropout rate because of IFN-related side effects is plotted in Figure 5. The onset of IFN-related side effects ranged from 204 to 1,569 days after initiation of IFN therapy. These side effects in nine patients disappeared 1 month after cessation of IFN therapy. None of the other patients developed serious side effects that required discontinuation of IFN.

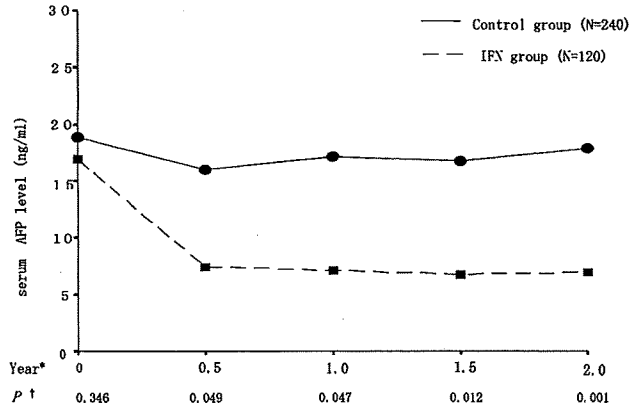

Fig. 2. Changes in serum AFP after initiation of treatment. *Year after initiation of treatment, †differences between IFN group and no-IFN group by the Krusukall Wallis test.

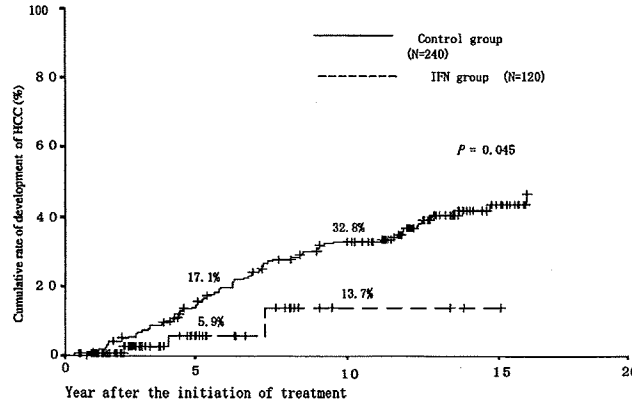

Fig. 3. Cumulative rate of development of hepatocellular carcinoma (HCC) based on the difference in treatment.

TABLE II. Factors Associated With the Development of HCC After Initiation of Treatment by Univariate Cox Regression Analysis

Factor	Category	Odds ratio	95% confidence interval	*P*-value
AFP after treatment (ng/ml)[a]	≤10	1	2.34–7.00	<0.0001
	>10	4.05		
Liver fibrosis	Mild or moderate	1	2.86–7.24	<0.0001
	Severe	4.55		
Sex	Male	1	0.34–0.84	0.006
	Female	0.532		
IFN therapy	−	1	0.13–0.98	0.045
	+	0.35		
AFP (ng/ml)[b]	≤10	1	0.45–36.90	0.209
	>10	2.23		
ALT after treatment[a]	≤50	1	0.53–6.29	0.342
	>50	1.82		
ALT (IU/L)[b]	≤100	1	0.73–2.34	0.371
	>100	1.31		
Age (years)[b]	≤65	1	0.23–11.78	0.615
	>65	1.65		
HCV-RNA after treatment[a]	−	1	0.29–14.88	0.474
	+	2.06		
BMI[b]	≤25	1	0.08–7.29	0.800
	>25	0.74		
HCV-RNA (KIU/ml)[b]	>100	1	0.05–4.21	0.490
	≤100	0.57		
HCV genotype[b]	1	1	0.45–2.49	0.907
	2	1.05		

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BMI, body mass index; HCC, hepatocellular carcinoma; IFN, interferon.
[a]Serum level 6 months after initiation of treatment.
[b]Measurement at initiation of treatment.

DISCUSSION

Several findings from the present study have direct implications for long-term IFN treatment in aged patients with chronic hepatitis or cirrhosis. First, the AFP baseline was decreased after initiation of IFN therapy in most patients. Second, the cumulative HCC development rate in patients whose serum level of AFP was within normal limits after initiation of IFN therapy was lower than that of patients with high level of AFP despite of IFN therapy. These suggest that AFP is a suitable indicator in long-term IFN therapy for protecting against HCC. If long-term IFN therapy could maintain normalization of serum AFP level, HCC development could be prevented in HCV patients.

AFP is a glycoprotein produced by the liver or yolk sac in fetal life in vertebrates, and it is not normally present in the serum of adults and is used commonly as a tumor marker for HCC [Otsuru et al., 1988]. Many reports have cited elevated AFP baselines as an independent HCC risk factor together with age, gender, liver histology stage, and ethnicity in patients infected with HCV [Ikeda et al., 1993; Tsukuma et al., 1993]. Elevation of AFP has been observed after a rise in transaminase in acute hepatitis, fulminant hepatitis, and acute exacerbation of chronic hepatitis. This type of AFP elevation is explained as a result of hepatocyte regeneration accompanied by necroinflammatory changes [Hu et al., 2004]. On the other hand, Yoshida et al. [2002] have reported that the HCV-coding core

TABLE III. Factors Associated With the Development of HCC After Initiation of Treatment by Multivariate Cox Regression Analysis

Factor	Category	Odds ratio	95% confidence interval	*P*-value
Liver fibrosis	Mild or moderate	1	1.69–6.13	<0.0001
	Severe	3.89		
AFP after treatment (ng/ml)[a]	≤10	1	1.45–4.84	0.002
	>10	2.65		
IFN therapy	−	1	0.11–0.86	0.025
	+	0.304		

AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; IFN, interferon.
[a]Serum AFP level 6 months after initiation of treatment.

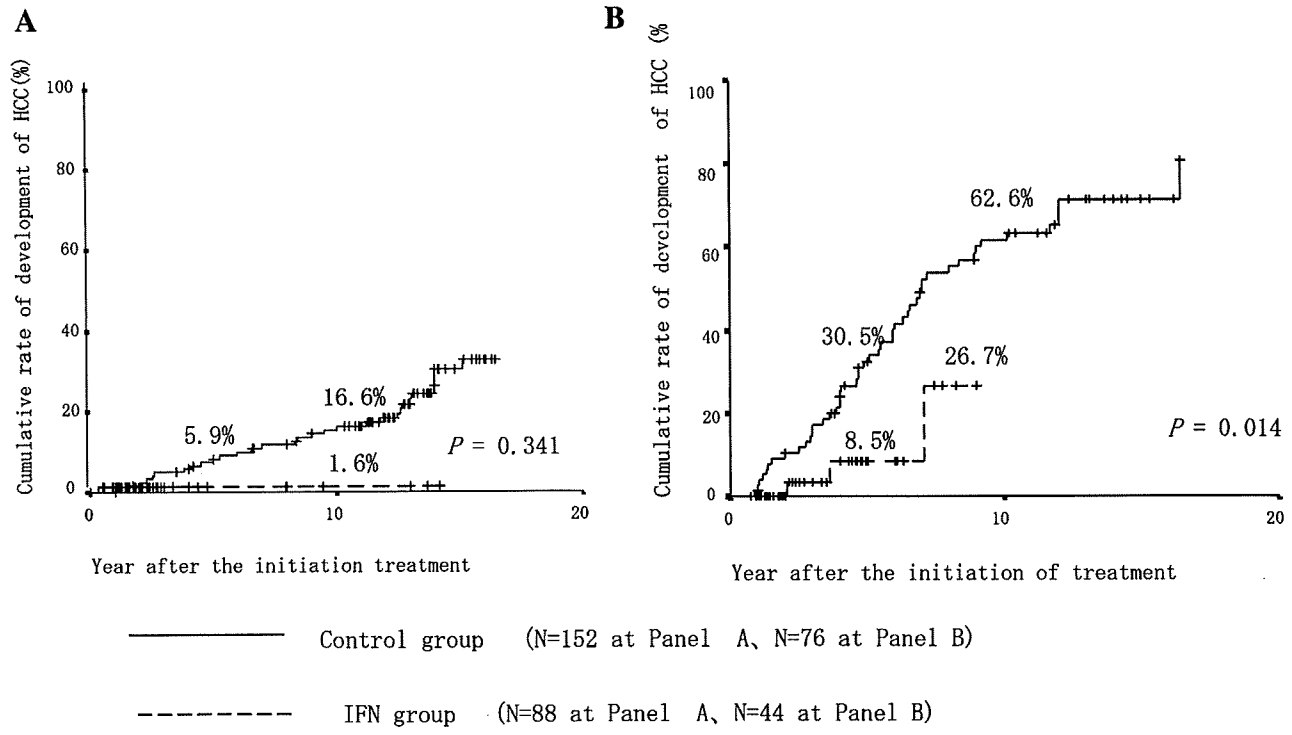

Fig. 4. Cumulative rate of development of hepatocellular carcinoma (HCC) based on the difference of treatment and histological staging (**Panel A**); patients with mild or moderate fibrosis. **Panel B**: patients with severe fibrosis.

protein is related to the cell cycle and cell proliferation at the transcriptional level in hepatocytes. This might mean that the HCV-coding core protein upregulate AFP production in hepatocytes.

AFP has the following functions associated with the hepatocarcinogenesis. First, AFP plays a role in the carrier-transport of various ligands and binds to a large variety of ligands such as fatty acids and estrogens. The action of AFP ligands can lead to cells toward multiplication or differentiation [Parmelee et al., 1978; Jacobson et al., 1990; Deutsch, 1991]. Second, AFP could control cell growth. AFP synergies growth factors such as epidermal growth factor and insulin like growth factor to cause proliferation of granulose cells. AFP has been found to regulate the proliferation of human mammary tumor cells [Wang and Alpert, 1995]. Wang and Xu [1998] have reported that human AFP can enhance the mouse hepatoma H-22 and human hepatoma SNMC-7721 cells in vitro. Third, AFP has been found to have immunosuppressive activity. AFP also suppresses the natural killer cell activity and induces suppressor T cells. AFP prevents the expression of MHC-II class molecule on macrophages [Lester et al., 1976].

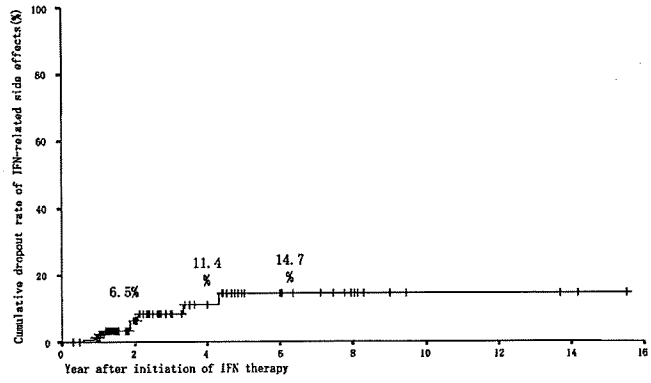

Fig. 5. Cumulative dropout rates due to IFN-related side effect.

Subsequently, Wang et al. [2001] has reported that antisense phosphorothioate oligodeoxyribonucleotide targeted to AFP genes inhibit the growth of human hepatoma cells and solid hepatoma, which is related to their cell apoptosis induction. As described above, elevated AFP might be associated with the hepatocarcinogenesis. Murashima et al. [2006] have reported that the competing action of IFN against HCV-related protein may cause decrease the production of AFP. Thus, normalization of serum AFP level by prolonged-IFN therapy could also protect against the development of HCC.

IFN therapy could reduce significantly the development of HCC in severe fibrosis. Long-term IFN treatment can be associated with serious side effects and is costly. Accordingly, careful selection of patients for long-term IFN therapy is important. The development rate of HCC is high in aged patients with severe liver fibrosis and elevated AFP. On the other hand, the development of HCC is low in no-aged patients with non-severe fibrosis and low level of AFP. Therefore, long-term IFN treatment for protection against HCC could be

recommended for patients with elevated AFP level and/or severe liver fibrosis who can tolerate IFN-related side effects. Considering cost-effectiveness, it seems reasonable to select aged patients with elevated AFP and/or severe fibrosis for long-term IFN therapy for protecting against the development of HCC.

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Short Communication

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

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

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

Results: In total, 20 (59%) patients experienced hepatitis reactivation. Although concentrations of HBV DNA and HBcrAg in serum did not differ between the two groups of patients at the onset of lamivudine administration, HBcrAg serum levels were significantly higher ($P = 0.009$) in the reactivation patients (median 4.9, 25–75% range 4.7–5.9 log unit/mL) than the non-reactivation patients (median 3.2, 25–75% range <3.0–4.5 log unit/mL) post-lamivudine treatment. The concentration of HBV DNA did not differ between the two groups (median <3.7, 25–75% range <3.7–<3.7 log copy/mL in the reactivation group vs. median <3.7, 25–75% range <3.7–<3.7 log copy/mL in the non-reactivation group). Receiver operating characteristic analysis of HBcrAg concentration showed an area under the curve of 0.764 in predicting patients without reactivation of hepatitis.

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

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

INTRODUCTION

LAMIVUDINE, A NUCLEOSIDE analog that inhibits reverse transcriptase, has been found to inhibit the replication of hepatitis B virus (HBV), reduce hepatitis, and improve histological findings of the liver in long-term treatment.[1,2] Furthermore, it has been shown that lamivudine treatment improves the long-term outcome of patients with chronic hepatitis B.[3,4] However, there are a number of problems with lamivudine therapy, including hepatitis relapse due to the appearance of YMDD mutant viruses and the reactivation of hepatitis after its discontinuation.[5,6]

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

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