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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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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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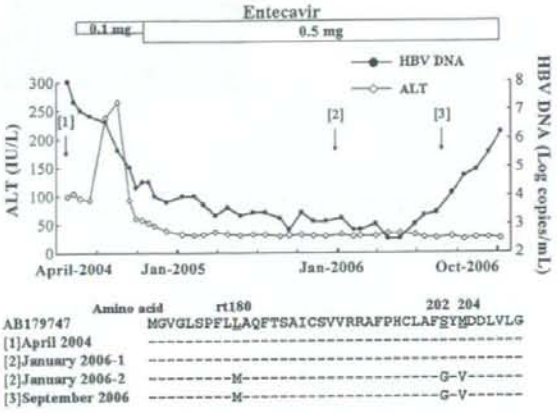

Fig. 1. Clinical course and evolution of viral polymerase reverse transcriptase gene sequence during the entecavir therapy. AB179747 was a strain reported by Ohnuma et al. (2005). In January 2006, there were two kinds of strains (January 2006-1 and -2).

(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.

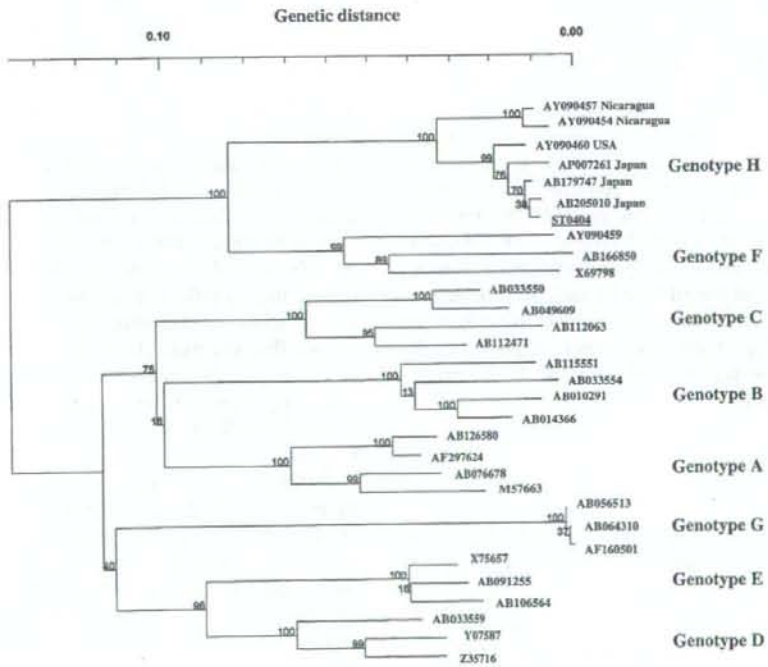

Fig. 2. Phylogram generated by neighbor-joining analysis of genetic distance in the full-length sequence of HBV. Thirty strains (without ST0404; indicated in underline) were retrieved from the GenBank/EMBL/DDBJ database.

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).

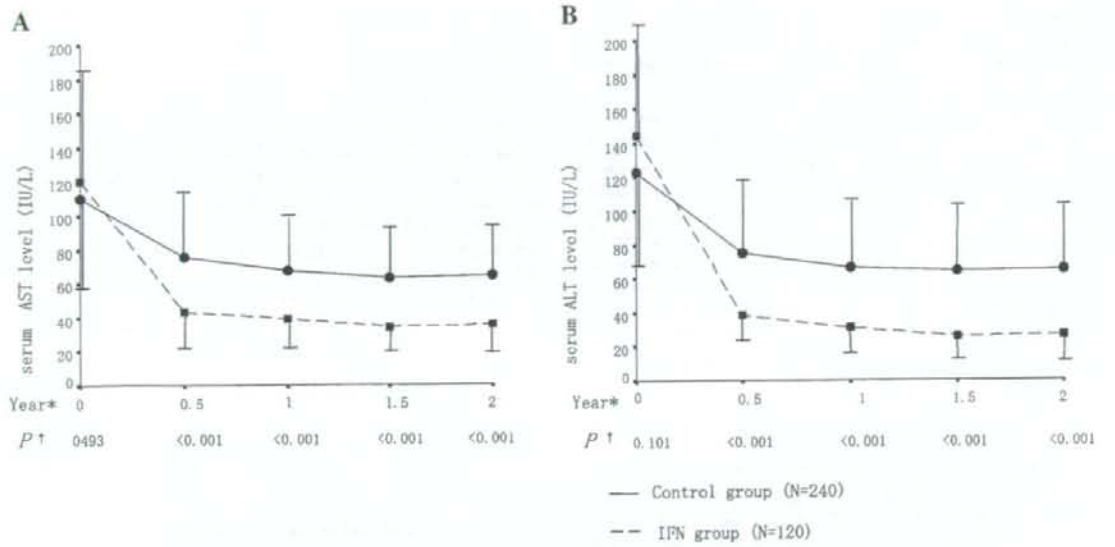

Fig. 1. Changes in serum AST (**panel A**) and ALT (**panel B**) after initiation of treatment. Data were expressed as mean ± standard deviation. *Year after initiation of treatment, †differences of both group by Krusukall Wallis test.

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.

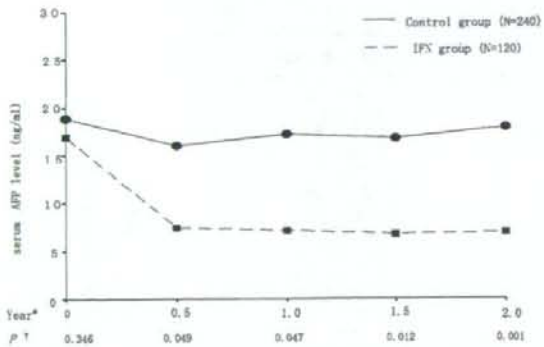

Fig. 2. Changes in serum AFP after initiation of treatment. *Year after initiation of treatment, †differences between IFN group and no-IFN group by the Krusukall Wallis test.

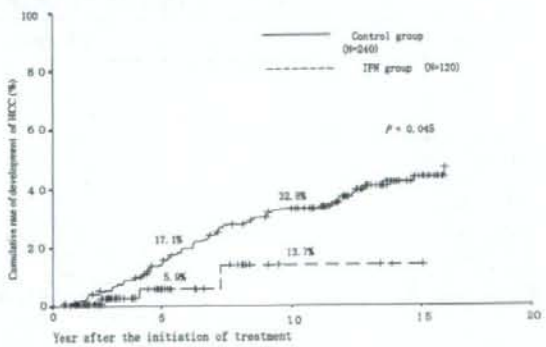

Fig. 3. Cumulative rate of development of hepatocellular carcinoma (HCC) based on the difference in treatment.

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.

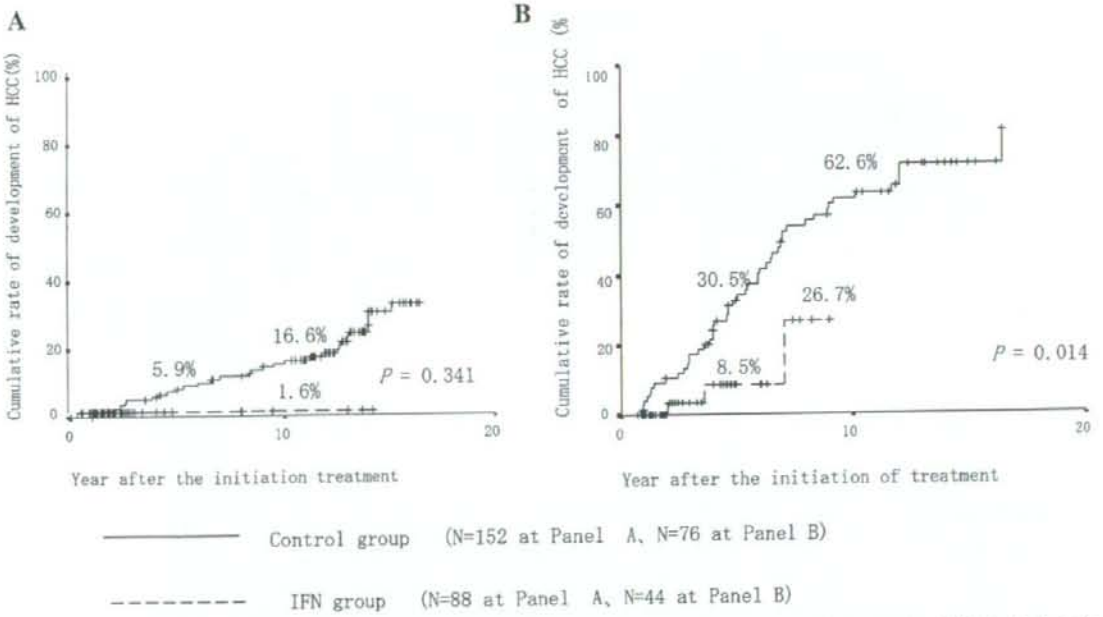

Fig. 4. Cumulative rate of development of hepatocellular carcinoma (HCC) based on the difference of treatment and histological staging (**Panel A**); patients with mild or moderate fibrosis. **Panel B**: patients with severe fibrosis.

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].

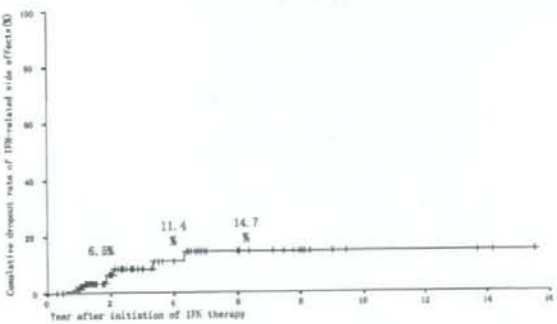

Fig. 5. Cumulative dropout rates due to IFN-related side effect.

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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administration as active hepatitis often recurs in patients post-treatment.

Previously, a chemiluminescence enzyme immunoassay (CLEIA) was developed by our laboratory to detect of hepatitis B core-related antigen (HBcrAg).[7,8] This HBcrAg CLEIA simultaneously measures the serum levels of hepatitis B core (HBc) and e (HBe) antigens using monoclonal antibodies, which recognize common epitopes of these two denatured antigens because both proteins are transcribed from the precore/core gene and their first 149 amino acids are identical.[9–11] Although this assay reflects the viral load of HBV in a similar manner to HBV DNA assays during disease progression, HBcrAg CLEIA shows characteristics different from HBV DNA assays under lamivudine administration since HBcrAg levels decrease more slowly than HBV DNA after treatment begins.[12] In the present study, we analyzed the clinical significance of the HBcrAg assay in predicting the likelihood of non-reactivation of hepatitis after discontinuing lamivudine administration in HBV treatment.

METHODS

Patients

A TOTAL OF 34 patients with chronic hepatitis B who were treated with lamivudine for at least 6 months were enrolled in the present study. The patients comprised 20 men and 14 women with a median age of 46 years (range 23–65 years), and were selected retrospectively from five medical institutions in Japan (Shinshu University Hospital, Kyoto Prefectural University Hospital, National Nagasaki Medical Center, Toranomon Hospital, and Hiroshima University Hospital). Written informed consent was obtained from each patient.

Of the 27 patients whose HBV genotype was determined, 25 (93%) were genotype C and the remaining two (7%) were genotype B. Serum HBV DNA was detectable in all patients, and HBe antigen was positive in 16 (47%) of the 34 patients before lamivudine administration.

For treatment of HBV infection, daily doses of 100 mg lamivudine were administered for at least 6 months. Lamivudine administration was stopped when alanine aminotransferase (ALT) levels were reduced to 40 IU/L or less in at least three separate tests. Serum samples were taken at several time points during and after lamivudine administration, and patients were seen at least once a month for at least 12 months after cessation of lamivudine. Estimated duration of HBV DNA level <3.7 log copy/mL before stopping lamivudine was a median 10 months (range 0–29 months).

Reactivation of hepatitis was defined as elevation of ALT to more than 80 IU/L within 12 months of stopping lamivudine treatment.

Serological markers for HBV

Serum hepatitis B surface antigen, HBe antigen, and anti-HBe antibody were measured by commercially available CLEIA kits (Fujirebio, Tokyo, Japan). Six major genotypes (A–F) of HBV are detectable using the method reported by Mizokami *et al.*[13] in which the surface gene sequence is amplified by polymerase chain reaction (PCR) and analyzed by restriction fragment length polymorphism. Serum concentration of HBV DNA was determined using a transcription mediated amplification (TMA) assay kit (Chugai Diagnostics Science, Tokyo, Japan) which has a quantitative range of 3.7–8.7 log copy/mL.

Serum concentration of HBcrAg was measured using a CLEIA developed by Fujirebio, as described previously.[7] Briefly, 150 μL of serum was incubated with 150 μL of pretreatment solution containing 15% sodium dodecylsulfate at 60°C for 30 min. After incubation, 120 μL of pretreated specimen was added to a ferrite microparticle solution in an assay tube. Ferrite microparticles were coated with monoclonal antibodies (HB44, HB61, HB114) against denatured HBc and HBe antigens. After washing, two other monoclonal antibodies against denatured HBcAg and HBeAg (HB91 and HB110) labeled with alkaline phosphatase were added as secondary antibodies. After further washing, 200 μL of AMPPD (3-(2′-spiroadamantan)-4-methoxy-4-(3″-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt; Applied Biosystems, Bedford, MA) solution was added as substrate, and the assay tube was incubated for 5 min at 37°C.

From this, the relative chemiluminescence intensity was measured, and HBcrAg concentration was determined by comparison with a standard curve generated using recombinant pro-HBe antigen (amino acids, 10–183 of the precore/core gene product). The HBcrAg concentration was expressed as units/mL (U/mL) and a immunoreactivity of recombinant pro-HBe antigen of 10 fg/mL was defined as 1 U/mL. In the present study, the cutoff value of HBcrAg concentration was set at 3.0 log U/mL.

Statistical analysis

The Mann–Whitney *U*-test was used to analyze quantitative data, and Fisher's exact test was used for

qualitative data. Receiver operating characteristic (ROC) curve analysis was used to analyze cut-off levels of HBcrAg concentration for prospective recurrence of hepatitis. Statistical analyses were performed using the SPSS 14.0 J statistical software package (SPSS, Chicago, IL, USA), and a *P*-value of less than 0.05 was considered to be statistically significant.

RESULTS

TWENTY (59%) OF the 34 patients enrolled in the present study showed reactivation of hepatitis within 12 months after discontinuing lamivudine administration, with 15 (75%) showing reactivation within 6 months. The peak serum ALT levels in the 20 reactivation patients ranged from 103 to 1019 IU/L, with a median of 308 IU/L. After lamivudine cessation, the maximum serum HBV DNA was significantly higher ($P < 0.001$) in the reactivation patients (median 7.8, 25–75% range 7.4–8.1 log copy/mL) than in the non-reactivation patients (median 4.8, 25–75% range 4.1–5.9 log copy/mL).

Table 1 shows a comparison of the clinical backgrounds at the onset and completion of lamivudine administration between the two groups of patients. Although backgrounds were similar between the two groups just prior to lamivudine administration, HBcrAg levels were significantly higher in the reactivation patients after treatment. Both HBV DNA levels and positive rates of HBe antigen were similarly low between the two groups. The duration of undetectable HBV DNA before stopping lamivudine administration was also similar ($P > 0.2$) between the two groups (reactivation patients, median 11 months, 25–75% range 8–13 months vs. non-reactivation patients, median 6 months, 25–75% range 5–13 months).

In 23 patients who were negative for HBe antigen after treatment, HBcrAg levels were significantly higher ($P = 0.011$) in the reactivation patients ($n = 12$, median 4.8 log U/mL, 25–75% range 4.0–5.0 log U/mL) than in non-reactivation patients ($n = 11$, median 3.0 log U/mL, 25–75% range 2.5–4.4 log U/mL). In contrast, levels were similar ($P > 0.2$) between the two groups in 11 patients who were positive for HBe antigen after treatment (reactivation patients $n = 8$, median 5.9 log U/mL, 25–75% range 5.1–6.1 log U/mL vs. non-reactivation patients $n = 3$, median 5.6 log U/mL, 25–75% range 2.5–8.0 log U/mL).

The ability of HBcrAg concentration to predict non-recurrence of hepatitis was analyzed using a ROC curve (Fig. 1), and the area under the curve was as wide as 0.764. The point at which specificity was 0.8 and sensi-

Table 1 Comparison of clinical characteristics at the onset and cessation of lamivudine administration between patients with and without reactivation of hepatitis

Characteristics	Reactivation of hepatitis		*P*-value†
	Positive ($n = 20$)	Negative ($n = 14$)	
Demographics			
Age (years)	44 (38–51)	50 (35–59)	NS
Sex (male/female)	13/7	7/7	NS
HBV genotype (B/C)	0/16	2/9	NS
At onset of lamivudine administration			
ALT (IU/mL)	103 (57–234)	211 (76–515)	NS
HBeAg (positive)	12 (60%)	4 (29%)	NS
HBV DNA (log copy/mL)	7.1 (6.1–8.1)	6.0 (5.3–7.4)	NS
HBcrAg (log unit/mL)	6.2 (5.6–7.7)	6.4 (5.0–6.6)	NS
At cessation of lamivudine administration			
Duration of lamivudine (months)	12.7 (10.4–16.3)	10.3 (6.4–17)	NS
ALT (IU/mL)	30 (15–36)	21 (15–24)	NS
HBeAg (positive)	8 (40%)	3 (21%)	NS
HBV DNA (log copy/mL)	<3.7 (<3.7–<3.7)	<3.7 (<3.7–<3.7)	NS
HBcrAg (log unit/mL)	4.9 (4.7–5.9)	3.2 (<3.0–4.5)	0.009

†Analysis of continuous variables performed using Mann–Whitney *U*-test; analysis of dichotomous variables performed using Fisher's exact test. Values shown as median (25–75% range) or *n* (%).
ALT, alanine aminotransferase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NS, not significant.

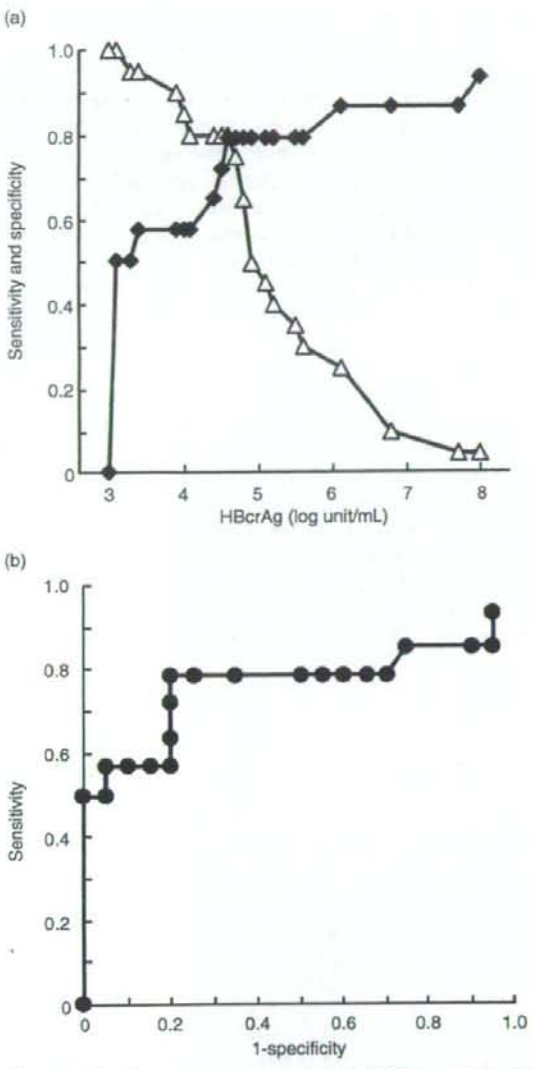

Figure 1 Receiver-operator characteristic (ROC) analysis of hepatitis B core-related antigen (HBcrAg) concentration for predicting patients without risk of reactivation of hepatitis within 12 months after halting lamivudine administration. (a) Sensitivity (■) and specificity (Δ) curves according to concentration of HBcrAg. (b) The ROC curve with the area under curve of 0.764.

tivity approximately 0.8 was deemed best for halting treatment without the risk of hepatitis recurrence. This point corresponds to an HBcrAg concentration of 4.1–4.6 log unit/mL.

DISCUSSION

THE REACTIVATION OF hepatitis following lamivudine administration was defined in the present study as an elevation of serum ALT level to more than 80 IU/L because we sought to find a more reliable indicator for safer discontinuation of lamivudine administration. Under these conditions, the majority (20/34) of patients showed reactivation of hepatitis within 12 months, as has been previously reported.[5,6] HBV DNA levels at the time of discontinuing lamivudine were similarly low between the two groups of patients, which is understandable as an undetectable reading typically indicates HBV remission following lamivudine therapy. However, HBcrAg levels were significantly higher in reactivation patients, implying that HBcrAg level is a better marker than HBV DNA level for predicting non-reactivation of hepatitis after discontinuing lamivudine administration especially in patients without HBe antigen.

In this study, ROC curve analyses showed a wide area under the curve of 0.764 in predicting the non-reactivation of HBV with HBcrAg level. If the corresponding cutoff is set at 4.5 logU/mL, then both specificity and sensitivity are as high as approximately 0.8. To obtain a higher specificity of 0.9, the cutoff value of HBcrAg concentration should be set at 4.0 log unit/mL. In this case, the sensitivity would still be nearly 0.6. The cutoff value of HBcrAg for predicting the non-relapse of hepatitis in our study is a little higher than that reported by Shinkai *et al.* (3.4 logU/mL).[14] Because numbers of patients analyzed were small in both studies, further studies are required to confirm the most appropriate cutoff value. It is noteworthy that this cutoff value may also differ among genotypes, which have been reported to be correlated with outcome of chronic HBV infection.[15] However, as over 90% of the patients had genotype C in this study, reactivation could not be analyzed in relation to HBV genotypes.

The HBV is an enveloped DNA virus containing a relaxed circular DNA genome which is converted into a covalently closed circular DNA (cccDNA) episome in the nucleus of infected cells and serves as transcriptional template for the production of viral RNA.[11,16,17] Reverse transcription of pregenomic RNA and second-strand DNA synthesis then occur in the cytoplasm within viral

capsids formed by the HBV core protein. Because lamivudine inhibits reverse transcription of pregenomic RNA, it directly suppresses production of HBV virions, and serum HBV DNA levels decrease rapidly after the initiation of lamivudine administration. However, the production of viral proteins is not suppressed by lamivudine as this process does not include reverse transcription. Furthermore, it has been reported that the amount of cccDNA, which also serves as a template for mRNAs, decreases quite slowly after commencement of administration of nucleoside analogs.[18,19] Thus, it is possible that serum HBcrAg levels reflect the cccDNA level in hepatocytes more accurately than serum HBV DNA. High levels of cccDNA are considered to be associated with hepatitis reactivation because they preceed reactivation of viral replication and consequent elevation of HBV DNA level in serum.

Lamivudine has already been eliminated from first line therapy in naïve chronic hepatitis B patients due to a higher incidence of developing resistant mutations than new antiviral agents, such as adefovir dipivoxil and entecavir.[20] However, the distinct characteristic of the HBcrAg assay under lamivudine therapy that is different from other HBV DNA assays is that lamivudine suppresses production of HBV virions by inhibiting reverse transcription of pregenomic RNA, but does not suppress the production of viral proteins, in which reverse transcription is unnecessary. Thus, it is possible that the HBcrAg assay may also useful for patients undergoing entecavir or adefovir dipivoxil administration because the main mechanism of suppressing HBV replication is similar between lamivudine and other antiviral agents. As a considerable number of patients who started lamivudine administration in the past are still taking this treatment now, the present study may be valuable for such patients when they consider changing therapies in the future. Additionally, further studies are required to determine whether the HBcrAg assay is indeed applicable to antiviral agents other than lamivudine.

In conclusion, significant markers that can predict reactivation of hepatitis after discontinuing lamivudine administration are clinically valuable because the reactivation of hepatitis is a fundamental problem in lamivudine therapy. Our results suggest that patients with an HBcrAg level of less than 4.5 log unit/mL may stop lamivudine administration with a lower risk of reactivation. The present study is a preliminary one because the patients enrolled were selected retrospectively without standardized criteria for stopping lamivudine and the number of patients enrolled was not large; however, the results may be valuable for patients with hepatitis B undergoing lamivudine therapy as such a diagnostic marker has rarely been reported. Further studies are required to establish the clinical significance of the HBcrAg assay in the treatment of hepatitis B.

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Interferon and lamivudine monotherapy on chronic hepatitis B in Japan

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Aim: We show data of interferon (IFN) and lamivudine monotherapy on chronic hepatitis B in Japan.

Methods: Data collected from sixty-six chronic hepatitis B (CHB) Japanese patients who were treated with IFN for 6 months were analyzed. The efficacy of long-term IFN therapy in 52 patients with e-antigen positive CHB, and data from 290 chronically HBV-infected patients who were treated with lamivudine for more than 3 years, were analyzed.

Results: Six-month IFN therapy: among 45 patients with HBeAg at commencement of IFN therapy, nine (20%) were responders. Young patients especially those with high serum alanine aminotransferase (ALT) levels were much more likely to respond to IFN therapy. Twelve-month IFN therapy: the response rate was 31% among 52 patients with HBeAg. Long-term lamivudine therapy: YMDD motif mutation was detected in 167 of 290 patients (58%) during lamivudine treatment. Breakthrough hepatitis from lamivudine resistant virus was detected in 93 of 290 patients (32%). Finally, 813 patients were treated by lamivudine between September 1995 and February 2006. Fifteen patients lost HBsAg during and after lamivudine therapy.

Conclusion: Long-term interferon therapy has a better response than short-term interferon therapy. Some patients lost HBsAg during and after lamivudine therapy.

Key words: HBV, interferon, lamivudine

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a common disease that can lead to a chronic carrier state, and is associated with risk of development of progressive disease and hepatocellular carcinoma.[1] Interferon (IFN) and lamivudine are two currently approved treatments for chronic hepatitis B (CHB) in most countries.[2] IFN is associated with significant adverse effects, and long-term therapy with lamivudine may result in drug resistance. A meta-analysis of IFN therapy published in 1993 reviewed 15 randomized controlled studies involving 837 adult patients who received IFN-α in doses of 5–10 million units (MU) administered daily to three times weekly for 4–6 months.[3] Loss of hepatitis B e-antigen (HBeAg) occurred in 33% of treated patients compared with 12% of controls. Loss of detectable HBV DNA and normalization of alanine aminotransferase (ALT) levels were also more common in treated than control patients. The main pretreatment factors that correlated with a response were high ALT levels,[4–6] low HBV DNA,[4,5] female sex, and greater degrees of activity and fibrosis on liver biopsy.[2] However, the optimal duration of IFN therapy for CHB is not well established. Moreover, the duration of IFN therapy was mainly one month in the 1990s in Japan and the efficacy was limited.[7–9]

Several studies have reported the effectiveness of some nucleoside analogs such as lamivudine[10–12] in the suppression of HBV replication, improvement of transaminase levels and liver histology, and enhancement of the rate of loss of HBeAg.[13] However, in patients who do not show loss of HBeAg, cessation of therapy after 3–12 months could potentially be associated with return to pretreatment HBV DNA levels and relapse of the disease.[14,15] Considering the safety of lamivudine, it has been suggested that continuous therapy may be beneficial, particularly in patients who do not show HBeAg seroconversion.[16] Leung *et al.*[17] showed that after 3 years of continuous treatment with lamivudine, 40% of patients achieved HBeAg seroconversion.

A large problem with long-term use of lamivudine, however, is the potential development of viral resistance, associated with increases in HBV DNA and serum transaminases. Resistance to lamivudine often develops after 6 months of treatment[18,19] and is associated with mutations in the HBV polymerase gene. Resistance was recently reported to develop in 15 and 38% of patients

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

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

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

INTERFERON THERAPY

Six-month IFN therapy

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

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

Twelve-month IFN therapy

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

LAMIVUDINE THERAPY

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