transcribed and translated in tandem, HBe antigen is produced and secreted into circulation [Bruss and Gerlich, 1988; Garcia et al., 1988]. The G to A mutation at nucleotide (nt) 1896 in the pre-C region (G1896A), which converts codon 28 for tryptophan to a stop codon, is associated with the loss of HBe antigen [Carman et al., 1989; Okamoto et al., 1990]. The double mutation (A1762T and G1764A) in the core promoter (CP) has been shown to reduce the synthesis of HBe antigen by suppressing the transcription of precore mRNA [Okamoto et al., 1994; Takahashi et al., 1995; Buckword et al., 1996]. Convincing lines of evidence have indicated a close association of HBe antigen seroconversion with the appearance of precore and core promoter mutations [Okamoto et al., 1994; Takahashi et al., 1995; Buckword et al., 1996; Yamaura et al., 2003] as well as the severity of liver disease [Kosaka et al., 1991; Aritomi et al., 1998; Lindh et al., 1998].

A chemiluminescence enzyme immunoassay (CLEIA) was developed previously for the detection of HBV core-related antigen [Kimura et al., 2002; Rokuhara et al., 2003]. The HBV core-related antigen is expressed on HBe and core (HBc) antigens; both proteins are transcribed from the precore/core gene and their first 149 amino acids are identical. The HBVcrAg CLEIA measures the serum levels of HBe and HBc antigens simultaneously, using monoclonal antibodies, which recognize common epitopes of these two denatured antigens. However, the amount of HBV core-related antigen mainly reflects that of HBe antigen, because the concentration of HBe antigen in serum is much higher than that of HBc antigen [Kimura et al., 2002]. In the present study, the characteristic differences that may exist between patients with and without HBV DNA in serum after HBe antigen seroconversion were examined by comparing chronological changes of HBV DNA and HBV core-related antigen as well as by testing HBV genome mutations associated with the seroconversion.

MATERIALS AND METHODS

Patients

The present study is a retrospective one using stored sera from Japanese patients with chronic hepatitis B seen in Shinshu University Hospital. The clinical database was reviewed to identify all patients who had been followed from January 1985 to June 2001 and also showed seroconversion from HBe antigen to anti-HBe during the follow-up period. A total of 24 patients were recruited in the present study. The 24 patients consisted of 17 men and 7 women with a median age of 39 years. Seroconversion of HBe antigen was defined as disappearance of HBe antigen accompanied by the development of anti-HBe on at least two consecutive visits. All 24 patients met the following three criteria: (1) follow-up was performed for at least 3 years before and after the seroconversion; (2) chronic hepatitis without liver cirrhosis was confirmed by histological examination; and (3) serum samples were available for testing every 6 months during the follow-up period. Of the 24 patients, 12 patients received interferon administration of at most 4 weeks and none received nucleotide analogs such as lamivudine, adefovir, or entecavir during the follow-up period.

Serum concentrations of HBV DNA and HBV core-related antigen were determined every 6 months during the follow-up period, which ran from 3 years before to 3 years after the seroconversion. The presence or absence of the pre-C mutation of A1896 and the double mutation in the CP (T1762/A1764) was determined every year during the follow-up period. The serum samples had been stored at −20°C or below until tested. Written informed consent was obtained from each patient.

Serological Markers for HBV

Conventional HBV markers, including HBe antigen and anti-HBe, were tested using CLEIA kits (Fuji Rebio, Tokyo, Japan). Six major genotypes (A–F) of HBV were determined using the method reported by Mizokami et al. [1999], in which the surface gene sequence amplified by PCR was analyzed by restriction fragment length polymorphism.

The Pre-C and CP mutations were determined on nucleic acids extracted from 100 μl of serum with a DNA/RNA extraction kit (Smitest EX-R and D; Genome Science Laboratories Co., Ltd., Tokyo, Japan). The stop codon mutation in the Pre-C region (A1896) was detected with an enzyme-linked mini-sequence assay kit (Smitest; Genome Science Laboratories). In principle, G1896 in the wild-type HBV and A1896 in the mutants were determined by mini-sequence reactions using labeled nucleotides that are complementary to either the wild-type or mutant. The results were expressed as a percent mutation rate according to the definition by Aritomi et al. [1998]. The sample was judged positive for the pre-C mutation when the mutation rate exceeded 50% in the present study, because the mutation rate steadily increase to 100% afterward once it exceed the rate of 50% [Yamaura et al., 2003]. The double mutation in the CP was detected using an HBV core promoter detection kit (Smitest; Genome Science Laboratories) [Aritomi et al., 1998]. This kit detects T1762/G1764 or A1762/T1764 by a polymerase chain reaction (PCR) with primers specific for either the wild-type or mutant. The results were recorded in three categories, that is, wild, mixed, and mutant types. In the present study, the sample was considered positive for the CP mutation when the results were in the mutant type category. The detection limits of the pre-C and the CP mutation kits are both 1,000 copies/ml according to the manufacturer. The pre-C mutation could be determined in 136 (99%) of 137 samples, which had HBV DNA levels higher than 1,000 copies/ml and in 30 (97%) of 31 samples which had levels lower than 1,000 copies/ml. Similarly, the CP mutation could be determined in 136 (99%) of 137 samples and in 28 (90%) of 31 samples.

The serum concentration of HBV DNA was determined using an Amplicor HBV monitor kit (Roche,

Tokyo, Japan) which had a quantitative range of 2.6–7.6 log copies/ml [Kessler et al., 1998]. Sera containing over 7.0 log copies/ml HBV DNA were diluted 10- or 100-fold in normal human serum and measured again to obtain the end titer.

The serum concentration of HBV core-related antigen was measured using the CLEIA reported previously [Kimura et al., 2002; Rokuhara et al., 2003]. In summary, 100 μl serum was mixed with 50 μl pretreatment solution containing 15% sodium dodecylsulfate and 2% Tween 60. After incubation at 70°C for 30 min, 50 μl pretreated serum was added to a well coated with monoclonal antibodies against denatured HBc and HBe antigens (HB44, HB61, and HB114) and filled with 100 μl assay buffer. The mixture was incubated for 2 hr at room temperature and the wells were washed with buffer. Alkaline phosphatase-labeled monoclonal antibodies against denatured HBc and HBe antigens (HB91 and HB110) were added to the well, and incubated for 1 hr at room temperature. After washing, CDP-Star with Emerald II (Applied Biosystems, Bedford, MA) was added and the plate was incubated for 20 min at room temperature. The relative chemiluminescence intensity was measured, and the HBV core-related antigen concentration was read by comparison to a standard curve generated using recombinant pro-HBe antigen (amino acids, 10–183 of the precore/core gene product). The HBV core-related antigen concentration was expressed as units/ml (U/ml) and the immunoreactivity of recombinant pro-HBe antigen at 10 fg/ml was defined as 1 U/ml. In the present study, the cut-off value was set tentatively at 3.0 log U/ml. Sera containing over 7.0 log U/ml HBV core-related antigen were diluted 10- or 100-fold in normal human serum and measured again to obtain the end titer.

Statistical Analyses

The Mann–Whitney U test was used to analyze continuous variables. The Fisher's exact test was used in the analysis of categorical data. The Manzel Haentel chi-square test was used to evaluate positive rates for the pre-C and CP mutations. The Wilcoxon test was used to analyze the change in the level of HBV DNA and HBV core-related antigen. *P*-values less than 0.05 were considered significant. Statistical analyses were performed using an SPSS 11.5 J statistical software package (SPSS, Inc., Chicago, IL).

RESULTS

Grouping of Seroconverters According to HBV DNA Outcome

The 24 seroconverters enrolled in the present study were classified into two groups according to changes in serum levels of HBV DNA. The HBV DNA level decreased substantially around the time of the seroconversion and then became continuously undetectable in one group (inactive replication group), and the level decreased slightly and did not become continuously undetectable even after the seroconversion in another group (active replication group). In the present study, the former group of patients were defined as those whose HBV DNA levels were lower than 2.6 log copies/ml at each of the time points of 1.5, 2, 2.5, and 3 years after the seroconversion, and the latter group of patients were defined as those whose HBV DNA levels were not. Of the 24 seroconverters, 6 belonged to the inactive replication group and the remaining 18 belonged to the active replication group.

The clinical backgrounds of the active and inactive replication groups are compared in Table I. The median age, gender ratio, and history of interferon therapy did not differ between the two groups. All patients were infected with genotype C HBV. Normalization of serum alanine aminotransferase (ALT) after seroconversion was considered to have occurred in cases in which ALT was normal at each of the time points of 2, 2.5, and 3 years after the seroconversion in the present study. The normalization of ALT was more frequent in the inactive replication group than in the active replication group, but the difference was not statistically significant.

Changes in HBV DNA and HBV Core-Related Antigen Concentration

Changes in the serum level of HBV DNA are compared between the active and inactive replication groups in Figure 1A. At the start-point of the follow-up, the level was distributed within a similarly high range in both groups. In the inactive replication group, the median

TABLE I. Comparison of Clinical Backgrounds Between the Inactive and Active Replication Groups

Characteristics	Inactive replication group n = 6	Active replication group n = 18	*P*
Age at seroconversion (yr)[a]	37 (23-65)	39 (17-64)	>0.2*
Gender (M:F)	4:2	13:5	>0.2**
Genotype C[b]	6 (100%)	18 (100%)	>0.2**
History of interferon therapy[b]	3 (50%)	9 (50%)	>0.2**
ALT normalization[c]	4 (67%)	5 (28%)	0.150**

*Mann–Whitney U test.
**Fisher's exact test.
[a]Data are expressed as the median (range).
[b]Data are expressed as a positive number (percent).
[c]Normalization of serum ALT level after seroconversion (the ALT value was within the normal range at each of the time points of 2, 2.5, and 3 years after the seroconversion).

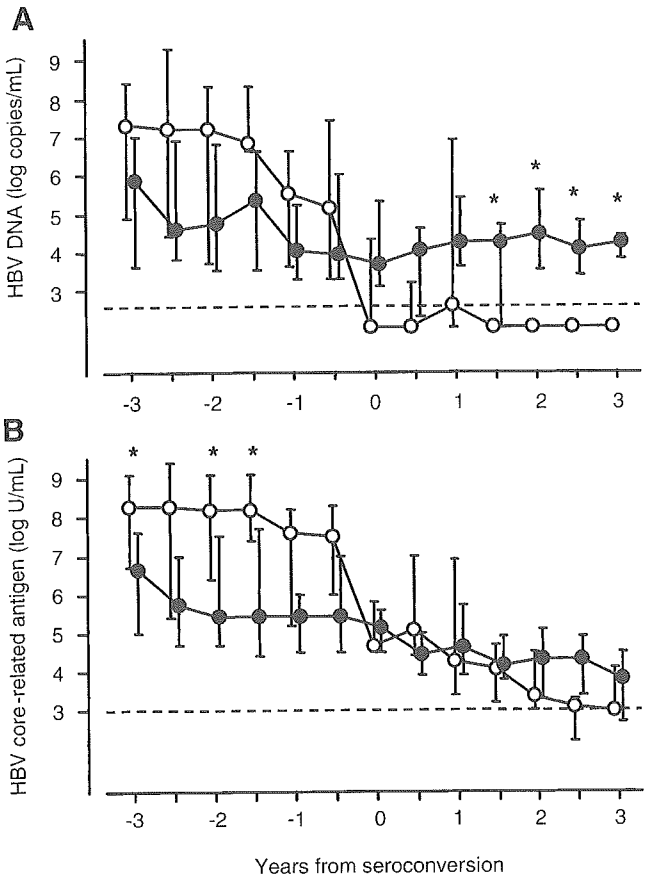

Fig. 1. Comparison of changes in HBV DNA (**A**) and HBV core-related antigen (**B**) levels between the inactive and active replication groups. Data are shown as the median ±25% ranges. The broken lines indicate the detection limits of the HBV DNA and HBV core-related antigen assays, respectively. Open circles indicate inactive replication group and closed circles indicate active replication group. $^*P < 0.05$ between the inactive and active replication groups.

concentration decreased around the time of seroconversion and became continuously undetectable thereafter. In the active replication group, on the other hand, the median concentration tended to decrease around the time of seroconversion, but was not undetectable even at 3 years after seroconversion. The median HBV DNA level in the active replication group was significantly higher than that in the inactive replication group at 1.5 years after the seroconversion and each of the subsequent time points.

Changes in the serum concentration of HBV core-related antigen are compared between the active and inactive replication groups in Figure 1B. The concentration of HBV core-related antigen was significantly higher in the inactive replication group than in the active replication group at the start of the follow-up and at 1.5 and 2 years before the seroconversion point. The median concentration of HBV core-related antigen in the inactive replication group appeared to decrease around the time of seroconversion and reached a level comparable to that in the active replication group. The median HBV core-related antigen level was similar between the inactive and active replication groups at all time points after the seroconversion, and it decreased slowly with time in both groups.

Changes in the log ratio of HBV core-related antigen/HBV DNA concentrations are compared between the inactive and active replication groups in Figure 2. The values of HBV core-related antigen and HBV DNA were substituted by their corresponding detection limit values when they were under the detection limit. The log ratio was similar between the two groups at points before the seroconversion. The log ratio decreased after the seroconversion in the active replication group, but did not change in the inactive replication group. The log ratio of HBV core-related antigen/HBV DNA was significantly lower in the active replication group than in the inactive replication group at all post-seroconversion time points except 1 year.

Comparison of Pre-C and CP Mutations

The positive rates for the pre-C and CP mutations at the time points before and after the seroconversion are compared between the inactive and active replication groups in Figure 3. The pre-C mutation did not appear during the follow-up period in the inactive replication group. On the other hand, the positive rate for the pre-C mutation was around 30% before the seroconversion, and then increased to around 60% after the seroconversion in the active replication group. The difference in the positive rate was significant at the time points of 2 and 3 years after the seroconversion. The positive rate for the CP mutation was less than 40% in the inactive replication group during the follow-up period except at the last time point, while it was over 60% in the active replication group throughout the follow-up period. The difference in the positive rate was statistically significant at the time points of 2 and 3 years before the seroconversion and at 1 and 2 years after it.

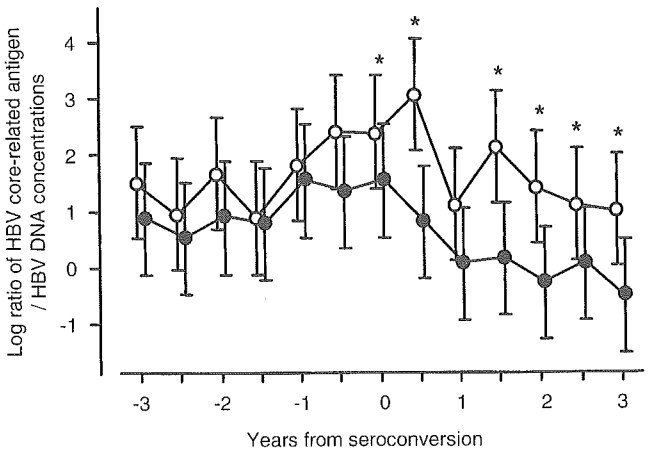

Fig. 2. Comparison of changes in the log ratio of HBV core-related antigen/HBV DNA levels between the inactive and active replication groups. Data are shown as the median ±25% ranges. Open circles indicate inactive replication group and closed circles indicate active replication group. $^*P < 0.05$ between the inactive and active replication groups.

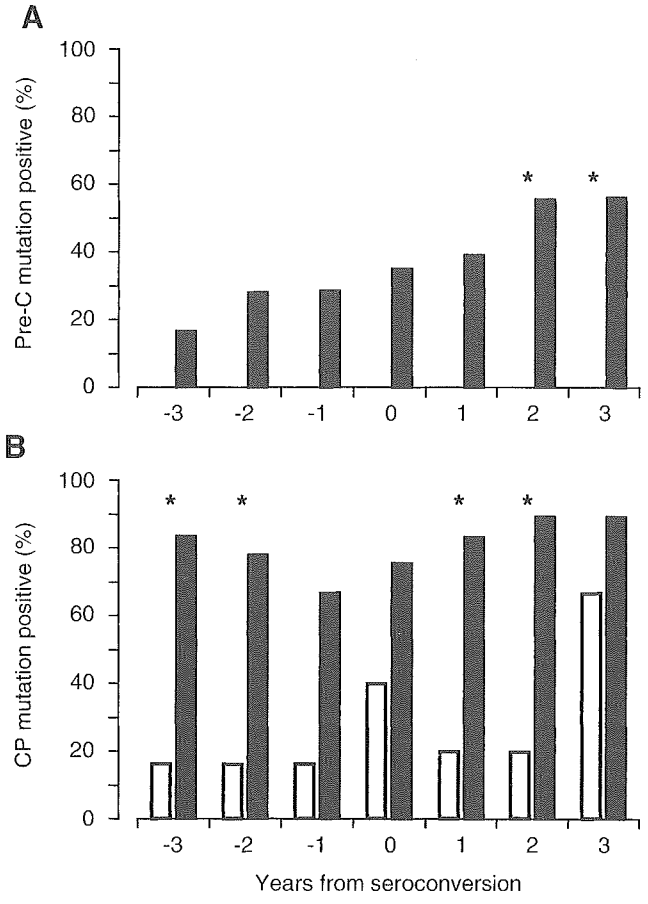

Fig. 3. Comparison of positive rates for the pre-C (**A**) and CP (**B**) mutations between the inactive and active replication groups. Open bars indicate inactive replication group and closed bars indicate active replication group. Number of patients in the inactive replication group is six at each time point except the followings: point 0 year (n = 5) in A, and points 0 year (n = 5), 1 year (n = 5), and 2 years (n = 5) in B. Number of patients in the active replication group is 18 at each time point except the followings: point 0 year (n = 17) in A and point 0 year (n = 17) in B. *$P < 0.05$ between the inactive and active replication groups.

DISCUSSION

Seroconverters were divided tentatively into two groups according to their levels of serum HBV DNA in the present study. It has been reported that older age and female gender are factors predicting occurrence of HBe antigen seroconversion in patients with chronic hepatitis B [Alward et al., 1985; Lok et al., 1987; McMahon et al., 2001]. On the other hand, in the present study, median age and gender distribution were similar between the inactive and active replication groups. A history of interferon treatment was recorded in half of the patients enrolled. The treatment history did not seem to be associated with the loss of HBV DNA after seroconversion, because the history was similarly distributed between the two groups and the duration of interferon therapy was as short as 4 weeks at most. Although the difference was not statistically significant, patients in the inactive replication group tended to show continuous normalization of ALT. Further, none of the six patients in the inactive replication group developed end stage liver diseases such as cirrhosis and hepatocellular carcinoma after the follow-up period, while 4 of the 18 patients in the active replication group developed them (data not shown). High viral load, which is usually associated with active hepatitis, has been reported to be a risk factor for development of hepatocellular carcinoma even in patients with chronic hepatitis B who achieved HBe antigen seroconversion [Ikeda et al., 2003; Ohata et al., 2004]. We could not compare long-term prognosis between patients in the inactive and active replication groups in the present study. However, patients in the active replication group tended to show active hepatitis after the seroconversion and to develop end stage liver diseases. Thus, further analysis of patients whose active viral replication continues after the seroconversion would be of clinical significance.

Analysis of the changes in HBV DNA and HBV core-related antigen revealed a clear contrast between the two. Namely, the HBV DNA level was similar between the two groups, while HBV core-related antigen was significantly lower in the active replication group than in the inactive replication group before seroconversion. The levels of both HBV DNA and HBV core-related antigen decreased remarkably around the time of seroconversion in the inactive replication group, while these levels did not change or decreased slightly in the active replication group. After seroconversion, the HBV DNA level was significantly higher in the active replication group than in the inactive replication group, while the HBV core-related antigen level was similar between the two groups. Because the discrepancy in the log ratio of HBV core-related antigen/ HBV DNA between the two groups first appeared at the time of seroconversion and continued thereafter, the difference between the HBV DNA and HBV core-related antigen changes was suggested to be closely associated with the seroconversion. The results obtained in the present study indicate that the mechanism of seroconversion was different between the two groups.

Because the serum level of HBV core-related antigen mainly reflects that of HBe antigen [Kimura et al., 2002], the low level of HBV core-related antigen seen after seroconversion in both the inactive and active replication groups might have contributed to the occurrence of seroconversion. The pre-C and CP mutations, which were associated with the seroconversion, were frequent in the active replication group and rare in the inactive replication group, at least at around the time of seroconversion. The decrease of HBV core-related antigen excretion seen after seroconversion was thought to have been caused mainly by the decrease of viral replication in the inactive replication group, because viral replication did not resume in this group. On the other hand, the decrease of HBV core-related antigen was thought to have been caused mainly by the appearance of pre-C and/or CP mutations, because active viral replication continued in this group. These results suggested that the two groups had different mechanisms of seroconversion.

It has been reported that the frequency of the pre-C and the CP mutations differs among HBV genotypes. Orito et al. reported that the CP mutation was significantly associated with genotype C [Orito et al., 2001]. Yamaura et al. [2003] reported that the CP mutation was already commonly seen several years before the seroconversion in patients with genotype C. These results are consistent with the present finding that the majority of patients in the active replication group had the CP mutation from the start of follow-up. The fact that patients in the active replication group had a lower level of HBV core-related antigen before the seroconversion may be attributable to the frequent CP mutation seen in this group.

In conclusion, the present study showed that there were different mechanisms of HBe antigen seroconversion between patients in whom HBV viraemia continued after the seroconversion and those in whom it did not. Measurement of HBV core-related antigen in addition to HBV DNA was suggested to be useful in examining specific conditions of chronic hepatitis B.

ACKNOWLEDGMENTS

We thank Ms. Toyo Amaki and Ms. Asami Yamazaki for their technical assistance. This research was supported in part by a Grant-in Aid from the Ministry of Health, Labour and Welfare of Japan (no. 1640013-41).

REFERENCES

Alward WL, McMahon BJ, Hall DB, Heyward WL, Francis DP, Bender TR. 1985. The long-term serological course of asymptomatic hepatitis B virus carriers and the development of primary hepatocellular carcinoma. J Infect Dis 151:604–609.

Aritomi T, Yatsuhashi H, Fujino T, Yamasaki K, Inoue O, Koga M, Kato Y, Yano M. 1998. Association of mutations in the core promoter and precore region of hepatitis virus with fulminant and severe acute hepatitis in Japan. J Gastroenterol Hepatol 13:1125–1132.

Bonino F, Rosina F, Rizzetto M, Rizzi R, Chiaberge E, Tardanico R, Callea F, Verme G. 1986. Chronic hepatitis in HBsAg carriers with serum HBV-DNA and anti-HBe. Gastroenterology 90:1268–1273.

Bruss V, Gerlich WH. 1988. Formation of transmembraneous hepatitis B e-antigen by cotranslational in vitro processing of the viral precore protein. Virology 163:268–275.

Buckword VE, Xu Z, Chen M, Yen TS, Ou JH. 1996. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. J Virol 70:5845–5851.

Carman WF, Jacyna MR, Hadziyannis S, Karayiannis P, McGarvey MJ, Makris A, Thomas HC. 1989. Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. Lancet 2:588–591.

Garcia PD, Ou JH, Rutter WJ, Walter P. 1988. Targeting of the hepatitis B virus precore protein to the endoplasmic reticulum membrane: After signal peptide cleavage translocation can be aborted and the product released into the cytoplasm. J Cell Biol 106:1093–1104.

Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. 1981. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Ann Intern Med 94:744–748.

Hsu YS, Chien RN, Yeh CT, Sheen IS, Chiou HY, Chu CM, Liaw YF. 2002. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology 35:1522–1527.

Ikeda K, Arase Y, Kobayashi M, Someya T, Saitoh S, Suzuki Y, Suzuki F, Tsubota A, Akuta N, Kumada H. 2003. Consistently low hepatitis B virus DNA saves patients from hepatocellular carcinogenesis in HBV-related cirrhosis. A nested case-control study using 96 untreated patients. Intervirology 46:96–104.

Kessler HH, Pierer K, Dragon E, Lackner H, Santner B, Stunzner D, Stelzl E, Waitzl B, Marth E. 1998. Evaluation of a new assay for HBV DNA quantitation in patients with chronic hepatitis B. Clin Diagn Virol 9:37–43.

Kimura T, Rokuhara A, Sakamoto Y, Yagi S, Tanaka E, Kiyosawa K, Maki N. 2002. Sensitive enzyme-immunoassay for hepatitis B virus core-related antigen and their correlation to virus load. J Clin Microbiol 40:439–445.

Kosaka Y, Takase K, Kojima M, Shimizu M, Inoue K, Yoshiba M, Tanaka S, Akahane Y, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. 1991. Fulminant HBV: Induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e-antigen. Gastroenterology 100:1087–1093.

Lee WM. 1997. Hepatitis B virus infection. N Engl J Med 337:1733–1745.

Liaw YF, Chu CM, Su IJ, Huang MJ, Lin DY, Chang-Chien CS. 1983. Clinical and histological events preceding hepatitis B e antigen seroconversion to antibody in chronic type B hepatitis. Gastroenterology 84:216–219.

Lindh M, Gustavson C, Mardberg K, Norkrans G, Dhillon AP, Horal P. 1998. Mutation of nucleotide 1762 in the core promoter region during hepatitis B e seroconversion and its relation to liver damage in hepatitis B e antigen carriers. J Med Virol 55:185–190.

Lok ASF, Lai CL, Wu PC, Leung EKY, Lam TS. 1987. Spontaneous hepatitis B e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. Gastroenterology 92:1839–1843.

Maddrey WC. 2000. Hepatitis B: An important public health issue. J Med Virol 61:362–366.

Maynard JE. 1990. . Hepatitis B: Global importance and need for control. Vaccine:S18–S320.

McMahon BJ, Holck P, Bulkow L, Snowball M. 2001. Serologic and clinical outcomes of 1536 Alaska natives chronically infected with hepatitis B virus. Ann Intern Med 135:759–768.

Mizokami M, Nakano T, Orito E, Tanaka Y, Sakugawa H, Mukaide M, Robertson BH, 1999. Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. FEBS Lett 450:66–71.

Ohata K, Hamasaki K, Toriyama K, Ishikawa H, Nakao K, Eguchi K. 2004. High viral load is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis B virus infection. J Gastroenterol Hepatol 19:670–675.

Okamoto H, Yotsumoto S, Akahane Y, Yamanaka T, Miyazaki Y, Sugai Y, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M. 1990. Hepatitis B viruses with precore region defects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen. J Virol 64:1298–1303.

Okamoto H, Tsuda F, Akahane Y, Sugai Y, Yoshiba M, Moriyama K, Tanaka T, Miyakawa Y, Mayumi M. 1994. Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. J Virol 68:8102–8110.

Orito E, Mizokami M, Sakugawa H, Michitaka K, Ishikawa K, Ichida T, Okanoue T, Yotsuyanagi H, Iino S. 2001. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Hepatology 33:218–223.

Realdi G, Alberti A, Rugge M, Bortolotti F, Rigoli AM, Tremolada F, Ruol A. 1980. Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B infection. Gastroenterology 79:195–199.

Rokuhara A, Tanaka E, Matsumoto A, Kimura T, Yamaura T, Orii K, Sun X, Yagi S, Maki N, Kiyosawa K. 2003. Clinical evaluation of a new enzyme immunoassay for hepatitis B virus core-related antigen; a marker distinct from viral DNA for monitoring lamivudine treatment. J Viral Hepatitis 10:1–7.

Takahashi K, Aoyama K, Ohno N, et al. 1995. The precore/core promoter mutant (T1762A1764) of hepatitis B virus: Clinical significance and an easy method for detection. J Gen Virol 76: 3159–3164.

Yamaura T, Tanaka E, Matsumoto A, Rokuhara A, Orii K, Yoshizawa K, Miyakawa Y, Kiyosawa K. 2003. A case-control study for early prediction of hepatitis B e antigen seroconversion by hepatitis B virus DNA Levels and mutations in the precore region and core promoter. J Med Virol 70:545–552.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Hepatology Research 32 (2005) 173–184
HEPATOLOGY RESEARCH
http://www.elsevier.com/locate/hepres

Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2795 patients

Akihiro Matsumoto [a], Eiji Tanaka [a,*], Akinori Rokuhara [a], Kendo Kiyosawa [a], Hiromitsu Kumada [b], Masao Omata [c], Kiwamu Okita [d], Norio Hayashi [e], Takeshi Okanoue [f], Shiro Iino [g], Kyuichi Tanikawa [h], the Inuyama Hepatitis Study Group [1]

[a] *Department of Internal Medicine, Gastroenterology and Hepatology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano-ken 390-8621, Japan*
[b] *Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan*
[c] *Department of Gastroenterology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan*
[d] *First Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Japan*
[e] *Department of Molecular Therapeutics, Osaka University Graduate School of Medicine, Osaka, Japan*
[f] *Department of Gastroenterology, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan*
[g] *Kiyokawa Hospital, Tokyo, Japan*
[h] *International Liver Research Institutes, Kurume, Japan*

Received 6 December 2004; received in revised form 7 February 2005; accepted 9 February 2005

Abstract

A retrospective survey of Japanese patients histologically diagnosed with chronic hepatitis B was conducted to determine the effectiveness of lamivudine in preventing hepatocellular carcinoma (HCC). Of the 2795 patients who satisfied criteria for analysis after treatment from any of 30 medical institutions, 657 had received lamivudine and the remaining 2138 had not. A Cox regression model with liver biopsy as the starting point revealed seven factors related to HCC: lamivudine therapy, gender, family clustering of hepatitis B, age at liver biopsy, hepatic fibrosis stage, serum albumin level, and platelet count. In a matched case-controlled study, 377 patients in a lamivudine-treated group and 377 matched patients in a non-treated group were selected based on their propensity scores. The mean follow-up period was 2.7 years in the lamivudine group and 5.3 years in the control group. In the lamivudine group, HCC occurred in four patients (1.1%) with an annual incidence rate of 0.4%/(patient/year), whereas in the control group HCC occurred in 50 patients (13.3%) for a rate of 2.5%/(patient/year). A comparison of the cumulative HCC incidence between the two groups by the Kaplan–Meier method showed a significantly lower incidence of HCC in the lamivudine group ($p < 0.001$). These findings suggest that lamivudine effectively reduces the incidence of HCC in patients with chronic hepatitis B.

Keywords: Chronic hepatitis B; Hepatocellular carcinoma; Anti-viral treatment; Lamivudine

1. Introduction

An estimated 350 million people worldwide are chronically infected with the hepatitis B virus (HBV), most in southeast Asia [1,2]. In this region, infection occurs during infancy, including that through mother–child transmission. Infected persons with HBV are initially asymptomatic, and

* Corresponding author. Tel.: +81 263 37 2633; fax: +81 263 32 9412.
E-mail address: etanaka@hsp.md.shinshu-u.ac.jp (E. Tanaka).
[1] Participating investigators are listed in Appendix A.

1386-6346/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.hepres.2005.02.006

active hepatitis emerges years later. In most patients, seroconversion from hepatitis Be antigen (HBeAg) to antibody to HBeAg (HBeAb) occurs spontaneously with age. At the same time, the virus levels decrease and hepatitis abates. Some patients, however, remain positive for HBeAg, and in those patients the hepatitis virus persists at high levels, resulting in the progression to hepatic cirrhosis, and the onset of hepatocellular carcinoma (HCC) in a high percentage of such patients [3–5]. The number of HBV carriers is decreasing in Japan and some other countries as a result of the prevention of mother–child transmission through the use of HBV vaccines and/or high-potency antibody to hepatitis B surface antigen (HBsAb) human immunoglobulin (HBIG) [6]. Even in these countries, however, only persons born after 1986 are protected by vaccination, and many chronic hepatitis B patients still need treatment. In the past, it was not easy to manage chronic hepatitis B using anti-viral agents such as interferon. In recent years, however, the development of lamivudine, a nucleoside analogue that inhibits reverse transcriptase, has drastically changed the treatment of hepatitis B [7–9]. By virtue of this inhibitory ability, lamivudine was developed as an anti-viral agent against human immuno-deficiency virus (HIV). It was later also found to be effective against HBV because HBV is a member of the Hepadnaviridae family, which utilizes reverse transcriptase in its replication process [10]. Lamivudine was found to inhibit the replication of HBV, reduce hepatitis, and improve liver histological findings in long-term treatment [11]. It is also useful when hepatitis B becomes severe due to acute exacerbation, as well as in the treatment of liver cirrhosis associated with symptoms of hepatic failure, such as ascites and edema [12–16]. However, a number of problems are associated with lamivudine therapy, such as relapse of hepatitis due to the appearance of YMDD mutant viruses and the difficulty of estimating the optimal time to discontinue the treatment [17,18]. In addition, until recently no adequate studies had been conducted to determine whether or not lamivudine inhibits the onset of hepatic cancer, even though it is known to slow the progression of histological changes in the liver. This lack of research is attributable partly to the need for long-term follow-up of a large number of patients and partly to the difficulty of conducting clinical trials. We conducted a multicenter study of a large number of registered patients to evaluate the effects of lamivudine on the course of hepatitis B and the onset of HCC. The data obtained were analyzed in a matched case-controlled study.

2. Materials and methods

2.1. Study design

The Inuyama Hepatitis Study Group designed this multicenter retrospective study to determine whether or not lamivudine is effective in preventing HCC. The subjects were Japanese patients with hepatitis B who were diagnosed with chronic liver disease by liver biopsy after 1980 and were followed up until March 2002. Each patient completed a questionnaire containing 16 items in four categories: background factors: date of birth, sex, family clustering of hepatitis B, and alcohol consumption during follow-up (80 g or more per day as ethanol); examination and test items: date of liver biopsy, grade and stage of histological findings of the liver, hepatitis Be antigen (HBeAg), antibody to HBeAg (HBeAb), albumin, asparate aminotransferase (AST), alanine aminotransferase (ALT), and platelet counts; clinical outcomes: the presence or absence of HCC during the follow-up period and the date of onset if present; lamivudine therapy: the presence or absence of lamivudine therapy during the follow-up period, and the date of initiation and duration of therapy if provided. The study was allowed by the review board of each participating institution. The names, ID numbers, and all other information that would directly identify individual patients were deleted to protect their privacy.

2.2. Patients

The present study included 3022 patients with chronic hepatitis B who underwent liver biopsy at any of 30 medical institutions after 1980. No patient had superinfection with hepatitis C virus and HIV. Two hundred and twenty-seven patients who had not answered the question about lamivudine treatment were excluded from the study. This left a total of 2795 patients for analysis. Among them, 657 patients had received lamivudine therapy and 2138 patients had not.

Histological findings of the liver were scored with respect to the grade of inflammation and stage of hepatic fibrosis according to the New Inuyama Histological Criteria [19] by a pathologist at each institution.

2.3. Lamivudine treatment

The lamivudine treatment group consisted of 657 patients who had received lamivudine therapy (100 mg/day). The median lamivudine treatment period was 18.9 months. Lamivudine therapy was continued until the end of the follow-up period in 45% of the patients.

2.4. Matched case-controlled study

In our analysis of the relationship between lamivudine therapy and hepatic carcinogenicity, the starting point was the day of liver biopsy. However, many patients in the lamivudine group (279 patients or 41.4%) initiated lamivudine therapy more than 2 years after liver biopsy, making them inappropriate subjects for the evaluation of the effects of lamivudine on hepatic carcinogenicity. For this reason, 377 patients who started lamivudine therapy within 2 years after liver biopsy were selected for analysis from the 657 patients in the lamivudine group. The interval from liver biopsy to lamivudine therapy was 5.8 ± 9.0 months, and the treatment

period was 23.1 ± 19.0 months (range 3–96 months). For the control group, seven factors were selected on the basis of the propensity scores from the 2138 patients who had not received lamivudine: age at the time of liver biopsy, gender, family clustering of hepatitis B, stage of hepatic fibrosis, serum albumin level, and platelet count. On that basis, 377 matching patients were selected for the control group [20].

2.5. Statistical analyses

A series of analyses was conducted using the day of liver biopsy as the starting point. Background factors at the time of liver biopsy were compared by the Student's *t*-test (numerical data) or the χ^2 test (categorical data), and differences were regarded as significant if $p < 0.05$ on both sides. Factors related to HCC were analyzed using a Cox regression model. The incidence of HCC was reported as an annual incidence rate (%/(patient/year)).

Because of the large differences in background factors between the lamivudine and control groups, the groups were matched for further analysis of HCC-related factors. For this analysis, all patients who had started lamivudine therapy within 2 years after liver biopsy were selected. The propensity score method was used to select patients from the control group [20]. Matching was done with respect to the HCC-related factors selected using the Cox regression model. After the matching, the incidence of HCC was shown by the Kaplan–Meier method and compared between the groups by the log-rank test. Differences were regarded as significant if $p < 0.05$ on both sides.

3. Results

3.1. Comparison of background factors

Table 1 demonstrates the comparison of background factors at the time of liver biopsy between the lamivudine and control groups. Significant differences were found in the mean age ($p < 0.001$), duration of follow-up ($p < 0.001$), history of IFN therapy ($p < 0.001$), inflammation of the liver ($p < 0.001$), HBeAg ($p < 0.001$), HBeAb ($p = 0.001$), serum albumin level ($p < 0.001$), AST level ($p = 0.011$), and platelet count ($p < 0.001$).

3.2. Evaluation of factors related to hepatic carcinogenicity by univariate analyses

HCC occurred in 31 of the 657 patients (4.7%) in the lamivudine group and in 239 of the 2138 patients (11.2%) in the control group. The mean follow-up periods after liver biopsy were 4.9 and 6.2 years in the lamivudine and control groups, respectively. Thus, the crude incidence of HCC determined was 1.0 and 1.8%/(patient/year) in the lamivudine and control groups, respectively.

Table 2 shows the incidences of HCC in the lamivudine and control groups in an analysis stratified with respect to background factors. In the lamivudine group, HCC did not occur in patients whose histological findings were grade 0 in inflammation and stage 0 in fibrosis, and significant intergroup differences were noted in this respect. No significant differences were observed other than in the histological findings.

3.3. Evaluation of factors related to hepatic carcinogenicity using a multivariate Cox regression model

Factors contributing to the incidence of HCC were analyzed using a Cox regression model (Table 3). The following variables were selected by the forward–backward stepwise selection method: lamivudine therapy (no therapy, $p = 0.002$), gender (male, $p < 0.001$), family history of hepatitis B (present, $p = 0.015$), age at the time of liver biopsy (older than 40 years, $p < 0.001$), stage of liver fibrosis (more than F2, $p < 0.001$), serum albumin level (less than 4.0 g/dL, $p = 0.001$), and platelet count (less than 150,000/μL, $p < 0.001$)). This analysis showed that lamivudine reduces the risk of HCC.

3.4. Evaluation of factors related to hepatic carcinogenicity by a six-factor matched case-controlled study

Matched case-control analyses were performed for six factors (sex, family history of hepatitis B, age at the time of liver biopsy, stage of liver fibrosis, serum albumin level, and platelet count). There were no significant differences in background factors between the groups, as shown in Table 4. The mean follow-up period in the control group (5.3 years) was about twice that in the lamivudine group (2.7 years). In the lamivudine group, HCC occurred in 4 of 377 patients (1.1%), with an annual incidence rate of 0.4%/(patient/year), compared to 50 of 377 patients (13.3%) and 2.5%/(patient/year), respectively, in the control group. A comparison of the cumulative HCC incidence between the two groups by the Kaplan–Meier method showed a significantly lower incidence in the lamivudine group ($p < 0.001$) (Fig. 1).

Next, the background factors were compared between patients with HCC and those without it in the lamivudine and control groups. In the lamivudine group (Table 5), the mean age was significantly higher in patients with HCC than in those without it (55.0 years versus 41.3 years, $p = 0.024$), but there were no significant differences in the other factors. In the control group (Table 6), the mean age was significantly higher in patients with HCC than in those without it (50.6 years versus 40.0 years, $p < 0.001$). Significant differences were also noted in the stage of liver fibrosis ($p < 0.001$), serum albumin level ($p < 0.001$), and platelet count ($p < 0.001$), suggesting that underlying liver disease was more advanced in patients who developed HCC.

Table 1
Comparison of background factors between lamivudine group and control group assessed at the time of liver biopsy

Parameter	Lamivudine group (n = 657)	Control group (n = 2138)	p-Value
Gender[a]			
Male	503 (76.6%)	1583 (74.0%)	0.194
Female	154 (23.4%)	555 (26.0%)	
Age (years)[b]	40.9 ± 11.0	37.3 ± 12.4	<0.001
Follow-up period (years)[b]	4.9 ± 4.4	6.2 ± 5.5	<0.001
Family clustering of hepatitis B[a]			
Yes	376 (57.2%)	1085 (50.7%)	0.011
No	242 (36.8%)	924 (43.2%)	
Unknown	39 (5.9%)	129 (6.0%)	
Drinking during the course of the study (>ethanol 80 g/day)			
Yes	69 (10.5%)	359 (16.8%)	<0.001
No	557 (84.8%)	1708 (79.9%)	
Unknown	31 (4.7%)	71 (3.3%)	
IFN therapy[a]			
Yes	269 (40.9%)	812 (38.0%)	<0.001
No	369 (56.2%)	1306 (61.1%)	
Unknown	19 (2.9%)	20 (0.9%)	
Liver histology			
Grade of inflammation[a]			
A0	15 (2.3%)	84 (3.9%)	<0.001
A1	194 (29.5%)	642 (30.0%)	
A2	283 (43.1%)	996 (46.6%)	
A3	142 (21.6%)	389 (18.2%)	
Unknown	23 (3.5%)	27 (1.3%)	
Stage of fibrosis[a]			
F0	12 (1.8%)	49 (2.3%)	0.491
F1	201 (30.6%)	721 (33.7%)	
F2	167 (25.4%)	524 (24.5%)	
F3	171 (26.0%)	491 (23.0%)	
F4	98 (14.9%)	331 (15.5%)	
Unknown	8 (1.2%)	22 (1.0%)	
HBeAg[a]			
+	355 (54.0%)	1272 (59.5%)	<0.001
–	280 (42.6%)	723 (33.8%)	
Unknown	22 (3.3%)	143 (6.7%)	
HBeAb[a]			
+	215 (32.7%)	642 (30.0%)	0.001
–	418 (63.6%)	1330 (62.2%)	
Unknown	24 (3.7%)	166 (7.8%)	
Albumin (g/dL)[b]	4.01 ± 0.49 (n = 629)	4.14 ± 0.49 (n = 1941)	<0.001
AST (IU/L)[b]	110.2 ± 131.8 (n = 593)	94.5 ± 131.5 (n = 2023)	0.011
ALT (IU/L)[b]	183.4 ± 211.1 (n = 641)	163.5 ± 234.3 (n = 2022)	0.056
Platelet count ($\times 1000/mm^3$)[b]	165.4 ± 54.9 (n = 629)	176.9 ± 59.6 (n = 1931)	<0.001

[a] Data are expressed as positive numbers (%).
[b] Data are expressed as means ± S.D.

4. Discussion

It is clear that this study has several limitations: it is not prospective, it is not randomized, there is no single regimen of lamivudine, and there is a lack of virological analysis (including that of the HBV genotype and that of YMDD mutations). It would be desirable to conduct a well-designed prospective study using controls. However, because lamivudine has been used in general practice under the insurance system in Japan, it is difficult to conduct a prospective and randomized control study of lamivudine therapy for chronic hepatitis B. In addition, it is ethically unacceptable to leave patients untreated for a long period of time in a control group, because lamivudine has been shown to abate hepatitis and improve histological findings of the liver [12–16].

Table 2
Comparison of the incidence of HCC in relation to each background factor between lamivudine group and control group

Parameter	Category	Group	Total number of patients (number)	No. of patients with HCC (number)	Average follow-up period (year)	Adjusted incidence of HCC (%/year)
Gender	Male	Lamivudine group	503	27	5.0	1.07
		Control group	1583	191	6.4	1.89
	Female	Lamivudine group	154	4	4.3	0.60
		Control group	555	48	5.6	1.54
Age (years)	<30	Lamivudine group	110	2	4.7	0.39
		Control group	642	8	5.9	0.21
	30≦ and <40	Lamivudine group	192	9	5.7	0.82
		Control group	646	52	6.8	1.18
	40≦ and <50	Lamivudine group	206	9	5.3	0.82
		Control group	491	75	6.7	2.28
	50≦	Lamivudine group	149	11	3.3	2.24
		Control group	359	104	5.3	5.47
Duration of lamivudine treatment (years)	<1	Lamivudine group	178	7	5.0	0.79
		Control group	–	–	–	–
	1≦ and <2	Lamivudine group	215	13	4.4	1.37
		Control group	–	–	–	–
	2≦ and <3	Lamivudine group	145	7	4.6	1.05
		Control group	–	–	–	–
	3≦	Lamivudine group	107	4	5.9	0.63
		Control group	–	–	–	–
Family clustering of hepatitis B	No	Lamivudine group	242	10	4.8	0.86
		Control group	924	100	6.4	1.69
	Yes	Lamivudine group	376	20	5.0	1.06
		Control group	1085	128	5.9	2.00
	Unknown	Lamivudine group	39	1	4.4	0.58
		Control group	129	11	8.2	1.04
Drinking during the course of the study (>ethanol 80 g/day)	No	Lamivudine group	557	23	4.8	0.86
		Control group	1708	158	5.8	1.59
	Yes	Lamivudine group	69	7	5.6	1.81
		Control group	359	76	7.8	2.71
	Unknown	Lamivudine group	31	1	3.8	0.85
		Control group	71	5	7.7	0.91
IFN therapy	No	Lamivudine group	369	19	4.2	1.23
		Control group	1306	167	6.0	2.13
	Yes	Lamivudine group	269	12	6.0	0.74
		Control group	812	70	6.5	1.33
	Unknown	Lamivudine group	19	0	2.6	0.00
		Control group	20	2	7.9	1.27
Liver histology Grade of inflammation	A0	Lamivudine group	15	0	9.3	0.00
		Control group	84	8	6.6	1.44
	A1	Lamivudine group	194	4	5.4	0.38
		Control group	642	59	6.4	1.44
	A2	Lamivudine group	283	15	4.9	1.08
		Control group	996	109	6.3	1.74
	A3	Lamivudine group	142	10	3.4	2.07
		Control group	389	52	5.5	2.43
	Unknown	Lamivudine group	23	2	6.1	1.43
		Control group	27	11	8.7	4.68

Table 2 (*Continued*)

Parameter	Category	Group	Total number of patients (number)	No. of patients with HCC (number)	Average follow-up period (year)	Adjusted incidence of HCC (%/year)
Stage of fibrosis	F0	Lamivudine group	12	0	7.2	0.00
		Control group	49	3	5.7	1.07
	F1	Lamivudine group	201	6	6.0	0.50
		Control group	721	29	6.7	0.60
	F2	Lamivudine group	167	8	4.7	1.02
		Control group	524	38	5.8	1.25
	F3	Lamivudine group	171	11	4.0	1.61
		Control group	491	61	6.0	2.07
	F4	Lamivudine group	98	6	3.6	1.70
		Control group	331	99	6.2	4.82
	Unknown	Lamivudine group	8	0	6.7	0.00
		Control group	22	9	8.3	4.93
HBeAg	–	Lamivudine group	280	10	4.2	0.85
		Control group	723	83	6.4	1.79
	+	Lamivudine group	355	19	5.3	1.01
		Control group	1272	134	6.0	1.76
	Unknown	Lamivudine group	22	2	6.2	1.47
		Control group	143	22	7.4	2.08
HBeAb	–	Lamivudine group	418	19	4.9	0.93
		Control group	1330	137	6.0	1.72
	+	Lamivudine group	215	10	4.7	0.99
		Control group	642	75	6.3	1.85
	Unknown	Lamivudine group	24	2	6.1	1.37
		Control group	166	27	7.4	2.20
Albumin (g/dL)	<4.0	Lamivudine group	257	19	4.5	1.64
		Control group	619	113	5.7	3.20
	4.0≦	Lamivudine group	372	9	4.9	0.49
		Control group	1322	90	6.1	1.12
AST (IU/L)	<50	Lamivudine group	187	7	5.7	0.66
		Control group	905	82	6.1	1.49
	50≦ and <100	Lamivudine group	200	14	4.7	1.49
		Control group	572	81	5.9	2.40
	100≦ and <200	Lamivudine group	142	7	5.1	0.97
		Control group	367	31	6.2	1.36
	200≦	Lamivudine group	64	2	4.4	0.71
		Control group	179	15	6.0	1.40
ALT (IU/L)	<50	Lamivudine group	117	5	4.7	0.91
		Control group	570	69	6.1	1.98
	50≦ and <100	Lamivudine group	155	7	4.9	0.92
		Control group	506	60	5.8	2.04
	100≦ and <150	Lamivudine group	109	9	4.7	1.76
		Control group	297	36	5.9	2.05
	150≦	Lamivudine group	260	9	4.8	0.72
		Control group	649	44	6.2	1.09
Platelet count ($\times 1000/mm^3$)	<150	Lamivudine group	254	18	3.8	1.86
		Control group	629	125	5.8	3.43
	150≦	Lamivudine group	375	11	5.3	0.55
		Control group	1302	67	6.1	0.84

Table 3
Estimation of effects of covariates following selection of regressor in Cox regression model

Category	Hazard ratio	95% Confidence interval (CI)	p-Value
Lamivudine therapy			
No	1		
Yes	0.49	0.31–0.77	0.002
Gender			
Male	1		
Female	0.42	0.28–0.62	<0.001
Family clustering of hepatitis B			
No	1		
Yes	1.44	1.08–1.94	0.015
Age at liver biopsy			
<40 y.o.	1		
≧40 y.o.	2.09	1.77–2.48	<0.001
Stage of liver fibrosis			
F0 or F1	1		
F2, F3, or F4	1.43	1.24–1.64	<0.001
Serum albumin level			
<4.0 g/dL	1		
≧4.0 g/dL	0.58	0.43–0.79	0.001
Platelet count			
<150 × 1000/μL	1		
≧150 × 1000/μL	0.53	0.38–0.73	<0.001

In the analysis of retrospective studies, great precautions are required in order to eliminate any bias between lamivudine-treated and non-treated groups. To minimize inter-group bias, we conducted with the cooperation of multiple medical institutions and a large number of patients ($n=2795$). The effect of lamivudine on HCC was ultimately analyzed in a matched case-controlled study. Because the time of liver biopsy was used as the starting point in our analysis, the analytical results were not expected to appropriately reflect lamivudine's effect if the therapy was started a long time after the biopsy. Therefore, from among the 657 patients who received lamivudine therapy, we selected 377 patients who started lamivudine therapy within 2 years after biopsy. For a control group, the same number of patients ($n=377$) without lamivudine therapy was selected from the 2138 subjects.

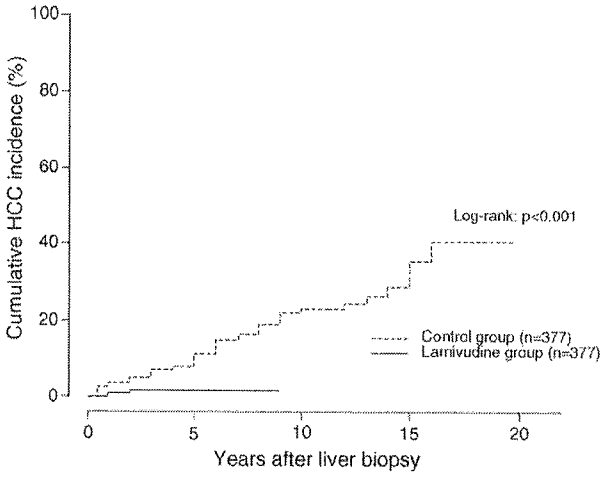

Fig. 1. Comparison of the cumulative HCC incidence between the lamivudine group (solid line) and the control group (broken line) by the Kaplan–Meier method in a case-matched control study. A significant difference was seen between the two groups ($p<0.001$, log-rank test).

The regimen was not the same in all patients who have been treated by lamivudine. It was transiently discontinued before being recommenced later in some patients, whereas it was uninterrupted throughout the follow-up period in the majority (63%) of subjects in the matched case-controlled study. The duration of lamivudine regimen was not taken into account in the design of our study. Some patients received lamivudine for relatively short periods to improve acute exacerbation of their clinical course in chronic hepatitis B. On the other hand, some patients received lamivudine for the long-term to suppress the development of HCC. In the analysis by a multivariate Cox regression model in all unmatched patients, lamivudine therapy was selected as one of the factors inhibiting the occurrence of HCC. In the matched case-controlled study, the annual occurrence rate of HCC was significantly lower (0.4%/(patient/year)) in the lamivudine group than in the control group (1.8%/(patient/year)), suggesting that lamivudine treatment is effective for inhibiting the occurrence of HCC.

Recently, Liaw et al. conducted a multicenter, centrally randomized, double-blind, placebo-controlled, parallel group study to evaluate the effects of lamivudine on the progression of chronic hepatitis B to hepatic cancer [21]. They randomized 651 patients with histologically confirmed (F3 and F4), compensated hepatic cirrhosis to receive either lamivudine or a placebo at a ratio of 2:1 and continued the treatment for up to 5 years. The study was terminated after a median treatment duration of 32.4 months (range 0–42) owing to a significant difference between the groups in the number of end points reached. The end points were reached by 7.8% of the patients receiving lamivudine and 17.7% of those receiving placebo (hazard ratio for disease progression, 0.45; $p=0.001$). The Child–Pugh score increased in 3.4% of the patients receiving lamivudine and in 8.8% of those receiving placebo (hazard ratio, 0.45; $p=0.02$), whereas HCC occurred in 3.9% of those in the lamivudine group and in 7.4% of those in the placebo group (hazard ratio, 0.49: $p=0.047$). The results of our analysis, which included patients with F0 through F2 hepatic fibrosis, were similar to those of Liaw et al. [21]. Thus, two studies demonstrated that the use of potent anti-viral agents such as lamivudine represents a major advance in the treatment of chronic hepatitis B and slows the progression of severe liver disease to liver cirrhosis as well as HCC.

Both hepatitis B and C are caused by persistent infection with hepatitis viruses, and both have a high probability of resulting in HCC. For this reason, these two diseases have a number of common traits, but some differences have been noted in their relationships with HCC. Among both

Table 4
Comparison of background factors between lamivudine group and control group assessed at the time of liver biopsy (matched case-controlled study)

Parameter	Lamivudine group ($n=377$)	Control group ($n=377$)	p-Value
Gender[a]			
Male	276 (73.2%)	273 (72.4%)	0.806
Female	101 (26.8%)	104 (27.6%)	
Age (years)[b]	41.5 ± 12.0	41.4 ± 12.2	0.950
Follow-up period (years)[b]	2.7 ± 2.1	5.3 ± 4.7	<0.001
Family clustering of hepatitis B[a]			
Yes	238 (63.1%)	242 (64.2%)	0.762
No	139 (36.9%)	135 (35.8%)	
Drinking during the course of the study (>ethanol 80 g/day)[a]			
Yes	38 (10.1%)	62 (16.4%)	0.007
No	333 (88.3%)	314 (83.3%)	
Unknown	6 (1.6%)	1 (0.3%)	
IFN therapy[a]			
Yes	129 (34.2%)	143 (37.9%)	0.046
No	236 (62.6%)	231 (61.3%)	
Unknown	12 (3.2%)	3 (0.8%)	
Liver histology			
Grade of inflammation[a]			
A0	6 (1.6%)	18 (4.8%)	0.001
A1	110 (29.2%)	101 (26.8%)	
A2	157 (41.6%)	186 (49.3%)	
A3	98 (26.0%)	72 (19.1%)	
Unknown	6 (1.6%)	0 (0.0%)	
Stage of fibrosis[a]			
F0	7 (1.9%)	6 (1.6%)	0.647
F1	103 (27.3%)	117 (31.0%)	
F2	95 (25.2%)	97 (25.7%)	
F3	107 (28.4%)	90 (23.9%)	
F4	65 (17.2%)	67 (17.8%)	
HBeAg[a]			
+	193 (51.2%)	220 (58.4%)	0.005
–	178 (47.2%)	141 (37.4%)	
Unknown	6 (1.6%)	16 (4.2%)	
HBeAb[a]			
+	126 (33.4%)	121 (32.1%)	0.030
–	245 (65.0%)	237 (62.9%)	
Unknown	6 (1.6%)	19 (5.0%)	
Albumin (g/dL)[b]	4.00 ± 0.51	4.00 ± 0.52	0.989
AST (IU/L)[b]	118.5 ± 155.4	95.5 ± 126.4	0.031
ALT (IU/L)[b]	191.7 ± 234.8	151.5 ± 180.5	0.009
Platelet count ($\times 1000/mm^3$)[b]	161.7 ± 52.7	164.3 ± 59.5	0.523

[a] Data are expressed as positive numbers (%).
[b] Data are expressed as means ± S.D.

hepatitis B patients and hepatitis C patients, HCC occurs mainly in those with advanced hepatic fibrosis, but the incidence of liver cirrhosis as a background of liver disease is lower in patients with B than in those with C. Furthermore, among hepatitis C patients HCC occurs mainly in those 60 years or older, while among hepatitis B patients it occurs mainly in those under 60 [22–24]. Studies on the cumulative incidence of HCC in hepatitis B patients showed that the HCC incidence increases linearly during the initial 12 years, plateaus, and then increases again in the 17th or 18th year [24,25]. In hepatitis C patients, on the other hand, the HCC incidence shows a continuous, linear increase [26,27]. Various findings obtained to date suggest that these clinical differences are related not only to differences in the hepatitis viral infection route and the timing of infection but also to differences in the mechanisms underlying cancer associated with hepatitis B and C. HCV is an RNA virus, and viral genes are not integrated into the host's genes, whereas HBV is a DNA virus with reverse-transcriptase activity. Thus, HBV genes are often integrated into the host's chromosomes

Table 5
Comparison of distribution of background factors between patients who developed HCC and those who did not in the lamivudine group (matched case-controlled study)

Parameter	Patients with HCC ($n=4$)	Patients without HCC ($n=373$)	p-Value
Gender[a]			
Male	3 (75.0%)	273 (73.2%)	1.000[c]
Female	1 (25.0%)	100 (26.8%)	
Age (years)[b]	55.0 ± 19.5 ($n=4$)	41.3 ± 11.9 ($n=373$)	0.024
Follow-up period (years)[b]	1.5 ± 0.6 ($n=4$)	2.7 ± 2.1 ($n=373$)	0.236
Family clustering of hepatitis B[a]			
Yes	2 (50.0%)	236 (63.3%)	0.628[c]
No	2 (50.0%)	137 (36.7%)	
Drinking during the course of the study (>ethanol 80 g/day)[a]			
Yes	1 (25.0%)	37 (9.9%)	0.393[c]
No	3 (75.0%)	330 (88.5%)	
Unknown	0 (0.0%)	6 (1.6%)	
IFN therapy[a]			
Yes	0 (0.0%)	129 (34.6%)	0.387[c]
No	4 (100.0%)	232 (62.2%)	
Unknown	0 (0.0%)	12 (3.2%)	
Liver histology			
Grade of inflammation[a]			
A0	0 (0.0%)	6 (1.6%)	0.458[c]
A1	0 (0.0%)	110 (29.5%)	
A2	3 (75.0%)	154 (41.3%)	
A3	1 (25.0%)	97 (26.0%)	
Unknown	0 (0.0%)	6 (1.6%)	
Stage of fibrosis[a]			
F0	0 (0.0%)	7 (1.9%)	0.918[c]
F1	1 (25.0%)	102 (27.3%)	
F2	1 (25.0%)	94 (25.2%)	
F3	2 (50.0%)	105 (28.2%)	
F4	0 (0.0%)	65 (17.4%)	
HBeAg[a]			
+	3 (75.0%)	190 (50.9%)	0.648[c]
−	1 (25.0%)	177 (47.5%)	
Unknown	0 (0.0%)	6 (1.6%)	
HBeAb[a]			
+	2 (50.0%)	124 (33.2%)	0.632[c]
−	2 (50.0%)	243 (65.1%)	
Unknown	0 (0.0%)	6 (1.6%)	
Albumin (g/dL)[b]	4.23 ± 0.45 ($n=4$)	4.00 ± 0.51 ($n=373$)	0.384
AST (IU/L)[b]	47.0 ± 22.8 ($n=4$)	119.4 ± 156.2 ($n=326$)	0.356
ALT (IU/L)[b]	46.3 ± 24.2 ($n=4$)	193.2 ± 235.5 ($n=372$)	0.213
Platelet count ($\times 1000/mm^3$)[b]	141.0 ± 27.0 ($n=4$)	161.9 ± 52.9 ($n=373$)	0.431

[a] Data are expressed as positive numbers (%).
[b] Data are expressed as means ± S.D.
[c] Fisher's exact test.

and play an important role in hepatic carcinogenesis [28,29]. It is known that the repeat of necrosis and regeneration of liver might accelerate the mutation of oncogenes. In addition, de novo carcinogenesis is thought to be promoted in hepatitis B patients as a result of the increased genetic instability caused by the integration of the HBV genome into the host's chromosomes. When administered to patients with hepatitis B, lamivudine decreases the blood HBV-DNA concentration and markedly improves ALT levels, with consequent improvement of liver histological findings [7,11,13,14]. An early in vitro study showed that lamivudine decreases the amount of free HBV-DNA in hepatocytes but does not affect integrated HBV genes [30]. Therefore, lamivudine is thought to inhibit HCC by abating hepatitis and not by inhibiting viral gene integration. In fact, as shown in the matched case control study, all four patients who developed HCC in the lamivudine group had non-cirrhotic liver disease, whereas 23 (46%) of 50 patients who developed HCC had liver cirrhosis. Due to the small number of patients included, however, further studies are necessary to confirm this finding.

Table 6
Comparison of distribution of background factors between patients who developed HCC and those who did not in the control group (matched case-controlled study)

Parameter	Patients with HCC ($n=50$)	Patients without HCC ($n=327$)	p-Value
Gender[a]			
Male	40 (80.0%)	233 (71.3%)	0.236[c]
Female	10 (20.0%)	94 (28.7%)	
Age (years)[b]	50.6 ± 10.1	40.0 ± 11.9	<0.001
Follow-up period (years)[b]	5.3 ± 4.3	5.2 ± 4.8	0.951
Family clustering of hepatitis B[a]			
Yes	29 (58.0%)	213 (65.1%)	0.345[c]
No	21 (42.0%)	114 (34.9%)	
Drinking during the course of the study (>ethanol 80 g/day)[a]			
Yes	14 (28.0%)	48 (14.7%)	0.050[c]
No	36 (72.0%)	278 (85.0%)	
Unknown	0 (0.0%)	1 (0.3%)	
IFN therapy[a]			
Yes	16 (32.0%)	127 (38.8%)	0.578[c]
No	34 (68.0%)	197 (60.2%)	
Unknown	0 (0.0%)	3 (0.9%)	
Liver histology			
Grade of inflammation[a]			
A0	2 (4.0%)	16 (4.9%)	0.026[c]
A1	6 (12.0%)	95 (29.1%)	
A2	27 (54.0%)	159 (48.6%)	
A3	15 (30.0%)	57 (17.4%)	
Stage of fibrosis[a]			
F0	0 (0.0%)	6 (1.8%)	<0.001[c]
F1	7 (14.0%)	110 (33.6%)	
F2	8 (16.0%)	89 (27.2%)	
F3	12 (24.0%)	78 (23.9%)	
F4	23 (46.0%)	44 (13.5%)	
HBeAg[a]			
+	26 (52.0%)	194 (59.3%)	0.564[c]
–	22 (44.0%)	119 (36.4%)	
Unknown	2 (4.0%)	14 (4.3%)	
HBeAb[a]			
+	20 (40.0%)	101 (30.9%)	0.319[c]
–	27 (54.0%)	210 (64.2%)	
Unknown	3 (6.0%)	16 (4.9%)	
Albumin (g/dL)[b]	3.63 ± 0.59	4.06 ± 0.49	<0.001
AST (IU/L)[b]	96.9 ± 100.8	95.3 ± 130.0	0.934
ALT (IU/L)[b]	132.8 ± 165.5	154.4 ± 182.7	0.431
Platelet count ($\times 1000/mm^3$)[b]	126.8 ± 50.7	170.0 ± 58.7	<0.001

[a] Data are expressed as positive numbers (%).
[b] Data are expressed as means ± S.D.
[c] Fisher's exact test.

Seven HBV genotypes (A–G) have been identified to date, and their distribution shows regional variations [31–36]. In Japan, genotypes C, B, and the other five account for 85, 12, and 3% of hepatitis B patients [36]. The virological differences between HBV genotype B and genotype C might influence not only on the natural course of hepatitis B but also the efficacy by lamivudine. The patients with HBV genotype B are frequently negative for HBeAg, have lower ALT levels and a better prognosis. In contrast, the patients with HBV genotype C tend to remain HBeAg-positive for a longer duration and tend to have elevated ALT levels and more advanced liver disease, such as liver cirrhosis and HCC. This indicates that the analysis of HBV genotypes will be needed in this study.

In conclusion, our multicenter, retrospective, matched case study indicated that lamivudine treatment might suppress the risk of HCC in patients with chronic hepatitis B. However, the study has several limitations, such as the relatively short duration of treatment and the lack of virological analyses (HBV genotype, YMDD mutation, and HBV-DNA volume). To relief these limitations, further long-term observation should be continued to clarify the conclusion.

Acknowledgment

This study was supported in part by a grant-in-aid from the Ministry of Health, Labor, and Welfare, Japan.

Appendix A

The Inuyama Hepatitis Study Group consists of the following 30 institutions and members: Dr. Sumio Watanabe (Akita University School of Medicine, Akita, Yamagata), Dr. Sumio Kawada (Yamagata University School of Medicine, Yamagata), Dr. Osamu Yokosuka (Chiba University, Graduate School of Medicine, Chiba), Dr. Kunihiko Hino (Delta Clinic, Tokorozawa), Dr. Hiromasa Ishii (Keio University, School of Medicine, Tokyo), Dr. Hiromitu Kumada (Toranomon Hospital, Tokyo), Dr. Gotaro Toda (Jikei University School of Medicine, Tokyo), Dr. Yasuyuki Arakawa (Nihon University School of Medicine, Tokyo), Dr. Nobuyuki Enomoto (Yamanashi University, School of Medicine, Kofu), Dr. Kendo Kiyosawa (Shinshu University School of Medicine, Matsumoto), Dr. Takafumi Ichida (Niigata University, Graduate School of Medical and Dental Science, Niigata), Dr. Tomoteru Kamimura (Niigata Saiseikai Hospital Dai-2, Niigata), Dr. Masashi Mizogami (Nagoya City University Graduate School of Medical Science, Nagoya), Dr. Shinichi Kakumu (Aichi Medical University, Nagoya), Dr. Hisataka Moriwaki (Gifu University School of Medicine, Gifu), Dr. Shuichi Kaneko (Kanazawa University, Graduate School of Medical Science, Kanazawa), Dr. Takeshi Okanoue (Kyoto Prefectural University, Graduate School of Medical Science, Kyoto), Dr. Norio Hayashi (Osaka University Graduate School of Medicine, Osaka), Dr. Masatoshi Kudo (Kinki University School of Medicine, Sayama), Dr. Yasushi Shiratori (Okayama University, Graduate School of Medicine and Dentist[r]y, Okayama), Dr. Gotaro Yamada (Kawasaki Hospital, Kawasaki Medical School, Okayama), Dr. Kazuaki Chayama (Hiroshima University, Graduate School of Biomedical Science, Hiroshima), Dr. Kiwamu Okita (Yamaguchi University, School of Medicine, Ube), Dr. Shigeki Kuriyama (Kagawa Medical University, Takamatsu), Dr. Morikazu Onji (Ehime University School of Medicine, Juushin-cho), Dr. Saburo Ohnishi (Kochi University School of Medicine, Nangoku), Dr. Michio Sata (Kurume University School of Medicine, Kurume), Dr. Shigetoshi Fujiyama, and Dr. Hiroshi Sasaki (Kumamoto University, Faculty of Medical and Pharmaceutical Science, Kumamoto), Dr. Hirohito Tsubouchi (Miyazaki University School of Medicine, Miyazaki), and Dr. Hiromi Ishibashi and Dr. Hiroshi Yatsuhashi (Nagasaki Medical Center, Omura).

References

[1] Lavanchy D. Hepatitis B virus epidemiology, disease burden treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97–107.
[2] Chen CJ, Wang LY, Wheiyu M. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. J Gastroenterol Hepatol 2000;15(Suppl):E3–6.
[3] Lok SF. Chronic hepatitis B. N Engl J Med 2002;346:1682–3.
[4] Chu CM. Natural history of chronic hepatitis B virus infection in adults with emphasis on the occurrence of cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol 2000;15(Suppl):E25–30.
[5] Tang B, Kruger WD, Chen G, et al. Hepatitis B viremia is associated with increased risk of hepatocellular carcinoma in chronic carriers. J Med Virol 2004;72:35–40.
[6] Oda T. Viral hepatitis and hepatocellular carcinoma prevention strategy in Japan. Jpn J Cancer Res 1999;90:1051–60.
[7] Dienstag JL, Perrillo RP, Schiff ER, et al. A preliminary trial of lamivudine for chronic hepatitis B infection. N Engl J Med 1995;333:1657–61.
[8] Nevens F, Main J, Honkoop P, et al. Lamivudine therapy for chronic hepatitis B: a six-month randomized dose-ranging study. Gastroenterology 1997;113:1258–63.
[9] Lai CL, Chien RN, Leung NW, et al. A one-year trial of lamivudine for chronic hepatitis B. N Engl J Med 1998;339:61–8.
[10] Severini A, Liu XY, Wilson JS, et al. Mechanism of inhibition of duck hepatitis B virus polymerase by (−)-beta-L-2′,3′-dideoxy-3′-thiacytidine. Antimicrob Agents Chemother 1995;39:1430–5.
[11] Ohkoshi S, Norio O, Ichida T. The long-term clinical outcome of 1-year treatment of chronic hepatitis B with lamivudine—5 years observation. Hepatol Res 2003;27:13–7.
[12] Leung NW, Lai CL, Chang TT, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis Be antigen seroconversion rates: results after 3 years of therapy. Hepatology 2001;33:1527–32.
[13] Lee HC, Suh DJ. Lamivudine therapy for decompensated liver cirrhosis related to hepatitis B virus infection. Intervirology 2003;46:388–93.
[14] Malekzadeh R, Mohamadhejad M. Reversibility of cirrhosis in chronic hepatitis B. Clin Gastroenterol Hepatol 2004;2:344–7.
[15] Fontana RJ, Hann HL, Perrillo RP, et al. Determinants of early mortality in patients with decompensated chronic hepatitis B treated with antiviral therapy. Gastroenterology 2002;123:719–27.
[16] Villeneuve JP, Condreay LD, Willems B, et al. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. Hepatology 2000;31:207–10.
[17] Lok AS, Hussain M, Cursano C, et al. Evolution of hepatitis B virus polymerase gene mutations in hepatitis E antigen-negative patients receiving lamivudine therapy. Hepatology 2000;32:1145–53.
[18] Fujioka S, Shimomura H. Two cases of chronic hepatitis B with emergence of lamivudine-resistant virus during long-term therapy. Hepatol Res 1999;12:97–104.
[19] Ichida F, Tsuji T, Omata M, et al. New Inuyama classification: new criteria for histological assessment of chronic hepatitis. Int Hepatol Com (Hepatol Res) 1996;6:112–9.
[20] D'agostino RB. Tutorial in biostatistics: propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. Stat Med 1998;17:2265–88.
[21] Liaw YF, Sung JJY, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521–31.
[22] Hamada H, Yatsuhashi H, Yano K, et al. Impact of aging on the development of hepatocellular carcinoma in patients with posttransfusion chronic hepatitis C. Cancer 2002;95:331–9.
[23] Ohnishi W, Kitamoto M, Aikata H, et al. Impact of aging on the development of hepatocellular carcinoma in patients with hepatitis C virus infection in Japan. Scand J Gastroenterol 2003;38:894–900.
[24] Kiyosawa K, Umemura T, Ichijo T, et al. Hepatocellular carcinoma: recent trends in Japan. Gastroenterology 2004;127:S17–26.
[25] Ikeda K, Saitoh S, Koida I, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observa-

tion of 795 patients with viral and alcoholic cirrhosis. Hepatology 2003;18:47–53.
[26] Ikeda K, Saitoh S, Suzuki Y, et al. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. J Hepatol 1998;28:930–8.
[27] Takano S, Yokosuka O, Imazeki F, et al. Incidence of hepatocellular carcinoma in chronic hepatitis B and C, a prospective study of 251 patients. Hepatology 1995;21:650–5.
[28] Buendia MA. Hepatitis B viruses and hepatocellular carcinoma. Adv Cancer Res 1992;59:167–226.
[29] Robinson WS. Molecular events in the pathogenesis of hepadnavirus-associated hepatocellular carcinoma. Ann Rev Med 1994; 45:297–323.
[30] Doong SL, Tsai CH, Schinazi RF, et al. Inhibition of the replication of hepatitis B virus in vitro by 2′,3′-dideoxy-3′-thiacytidine and related analogues. Proc Natl Acad Sci USA 1991;88:8495–9.
[31] Miyakawa Y, Mizokami M. Classifying hepatitis B virus genotype. Intervirology 2003;46:329–38.
[32] Orito E, Ichida T, Sakugawa H, et al. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology 2001;34:590–4.
[33] Kao JH, Chen PJ, Lai MY, et al. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000;118:554–9.
[34] Sugauchi F, Kumada H, Acharya SA, et al. Epidemiological and sequence differences between two subtypes (Ae and Aa) of hepatitis B virus genotype A. J Gen Virol 2004;85:811–20.
[35] Orito E, Mizokami M. Hepatitis B virus genotypes and hepatocellular carcinoma in Japan. Intervirology 2003;46:408–12.
[36] Orito E, Ichida T, Sakugawa H, et al. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology 2001;34:590–4.

肝疾患の病態と対策

特集

B型慢性肝炎治療の最前線 インターフェロン療法

Interferon therapy for chronic hepatitis B

加藤 道夫
KATO Michio

肝臓の臨床最前線

Key words　IFN治療　HBステージ分類　IFN・ラミブジン併用治療　ペグIFN治療　長期予後

HBVキャリアはHBe抗原陽性無症候性キャリアから慢性肝炎，肝硬変，肝細胞癌あるいは臨床的治癒とされているHBe抗体陽性無症候性キャリアまでさまざまな病態が存在する．そして，その経過もさまざまであるが，大別すると肝硬変，肝細胞癌に進行する群と臨床的治癒の状態に落ち着く群に二分される．約80%は後者になると考えられるが，B型肝癌も全肝細胞癌中10～15%を占め，現在，死亡者数は横ばいで年間約5,000名を数えている．これに，肝不全死や劇症化による死亡を加えるとB型肝疾患による死亡者数は年間7,000名～8,000名に上ると考えられる．B型慢性肝炎の予後改善にはHBe抗原の陰性化とHBV-DNAの低値持続が必要であり，そのためには適切な抗ウィルス治療が肝要となる．現在，インターフェロン(IFN)，ラミブジンおよびアデホビルが保険適用製剤であるが，本稿ではB型慢性肝炎に対するIFN治療の現状と今後の展望について述べる．

Ⅰ．HBVキャリアのステージ分類とIFN治療対象の位置づけ

われわれはB型慢性肝炎の肝硬変進展，肝癌発癌抑止を目的とした適切な抗ウィルス治療の選択に向けての，HBVマーカーと発癌リスクよりみたHBVキャリアのステージ分類を提唱した[1]（表1）．

独立行政法人大阪医療センター消化器科　部長

HBステージ0

HBs抗原陽性，HBe抗原陽性，ALT正常値持続のいわゆる無症候性キャリアの状態．発癌リスクはほとんどなく，抗ウィルス治療の適応なし．

HBステージⅠ

HBs抗原陽性，HBe抗原陽性，ALT異常値(持続正常以外)でHBV-DNA量が$10^{7.6}$copies/mL以上の高ウィルス群．若年例(男性：30歳未満，女性：35歳未満)をステージⅠa，高年例(男性：30歳以上，女性：35歳以上)をステージⅠbとする．ステージ1a群も発癌リスクがきわめてまれで，

0371-1900/05/¥50/頁/JCLS

表1 HBV キャリアのステージ分類

HB stage	0	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ
HBsAg	＋	＋	＋	＋	＋	**－
HBeAg	＋	＋	＋	－	－	－
HBV-DNA (copies/ml)	不問	$10^{7.6}$≦	$10^{7.6}$＞	10^{5}≦	10^{5}＞	不問
ALT	持続正常	*持続正常以外	*持続正常以外	不問	不問	不問
年齢	不問	若年/高年 (Ⅰa/Ⅰb)	若年/高年 (Ⅱa/Ⅱb)	不問	不問	不問
発癌リスク	きわめて小	小/大	小/きわめて大	きわめて大	きわめて小	きわめて小

*若年：男性30歳未満，女性35歳未満　** HBsAg(＋)の時期が確認されていること
高年：男性30歳以上，女性35歳以上

通常は抗ウィルス治療の必要はないが組織学的に線維化ステージが進行している例は抗ウィルス治療の適応となる．ステージⅠa，Ⅱaとも薬剤としては若年で免疫応答が良好であるのでIFNが第一選択となると考える．IFNについてわれわれは後述の少量間歇投与が若年例に有効であることを報告[2]したが，特に30歳未満例にはIFN治療は長期投与でなくても有効性は高いと考える．一方，ステージⅠb群は発癌リスクを有し，抗ウィルス治療の必要を認める．HBV-DNA量がきわめて高値のこの群はラミブジン単独での治療効果の持続は困難で，エンテカビル等の抗ウィルス効果の強い薬剤あるいはIFN/ラミブジン併用治療が適応になると考えられる．

HB ステージⅡ

HBs抗原陽性，HBe抗原陽性，ALT異常値(持続正常以外)でHBV-DNA量が$10^{7.6}$copies/mL未満の低ウィルス群．若年例をステージⅡa，高年例をステージⅡbとする．ステージⅡa群は発癌リスクは少ないが若年発癌例が存在し，またALT高値が持続する例も多く，抗ウィルス治療の適応になる．ステージⅡb群は発癌リスクがきわめて大で抗ウィルス治療の絶対適応である．ラミブジン等の核酸アナログ単独あるいはIFN，HBワクチンとの併用の選択が考えられる．

HB ステージⅢ

HBs抗原陽性，HBe抗原陰性，HBV-DNA 10^{5}copies/mL以上のpre-core mutant株のreplicationが持続している群である．発癌リスクはきわめて大で，ALT値異常のとくに男性はステージⅡbとともに抗ウィルス治療の絶対適応である．薬剤としては高年例が大半を占め，ラミブジンの治療効果が良好でYMDD変異株の出現も低率であるため，現在のところラミブジンが第一選択であり，YMDD変異株出現例にはアデホビル等の他の核酸アナログの併用あるいは切り替えで対応できると考えられる．

HB ステージⅣ

HBs抗原陽性，HBe抗原陰性，HBV-DNA 10^{5}copies/mL未満のいわゆる臨床的治癒の状態である．発癌リスクとしてはきわめてまれで原則的には抗ウィルス治療の必要はないと考える．

HB ステージⅤ

HBキャリア(HBs抗原陽性の時期が確認されている例)でHBs抗原が消失した状態である．HBステージⅣと同様，発癌リスクはきわめてまれで抗ウィルス治療の必要はない．

HBVキャリアの大多数が歩む臨床的治癒状態へのコースはステージⅠaからステージⅡaとなり，その後短期間ステージⅢを経由した後速やかにステージⅣに移行するものと考えられる．そしてステージⅣが長期間続いた後HBs抗原が消失し，ステージⅤとなる．一方，肝硬変進展・肝癌発癌ハイリスク群はステージⅠaからステージ

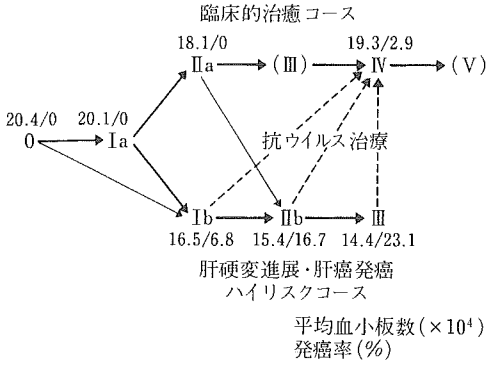

図1　**HBV** キャリアの経過(臨床的治癒コースと肝硬変進展・肝癌発癌ハイリスクコース)

Ⅰb，ステージⅡbと進行し，HBe抗原が陰性化してステージⅢまでは到達するがHBVの増殖は持続し，ステージⅣに至ることはない(**図1**)．臨床的治癒コースの各ステージにおける初診時の血小板数と発癌リスクは，ステージ0，Ⅰa，ⅡaおよびⅣでそれぞれ20.4万/0%，20.1万/0%，18.1万/0%および19.3万/2.9%とほとんど変化を認めないが，肝硬変進展・肝癌発癌ハイリスクコースにあたるステージⅠb，Ⅱbおよびでは16.5万/6.8%，15.4万/16.7%および14.4万/23.1%とステージの移行にしたがっての血小板数の低下と発癌率の増加が認められ，ステージⅠb，ⅡbおよびⅢのキャリアに対する抗ウィルス治療の必要性が強く示唆される．特にIFN治療の適用対象としては，HBe抗原陽性若年例(ステージⅠa，Ⅱa)ではIFN単独，HBe抗原陽性高年例(ステージⅠb，Ⅱb)ではIFN/ラミブジン併用治療(後述)が適切な選択ではないかと考える．

Ⅱ．これまでのIFN治療

1．少量間欠投与

われわれは1984年よりHBe抗原陽性例に対してnatural IFNαの少量間欠投与を行い，良好な成績を報告[2]した．投与法は大阪府赤十字血液センターより供与をうけたヒト白血球IFNを週1回計4回(初回量2.4MU～3MU，以下漸減投与)総量6.8MU～10MUの投与である．投与対象の性別は男性9例，女性6例で，それぞれの年齢は男性20歳～55歳(平均35.8歳)，女性22歳～45歳(平均31.7歳)であった．成績は投与終了後6ヵ月でのHBe抗原陰性化率53.3%(**図2**)，HBe抗原抗体seroconversion率33.3%およびALT正常化率53.3%といずれも高率であった．HBe抗原陰性化例の多くは投与終了後，ALTの上昇後に消失し，女性，HBe抗原価低値，投与前ALT高値および組織診断でactivityの高い症例に得られやすいことが判った．対象の60%が35歳未満の若年であったことが良好な成績が得られた要因と考えられ，HBステージⅠa，Ⅱaで抗ウィルス治療

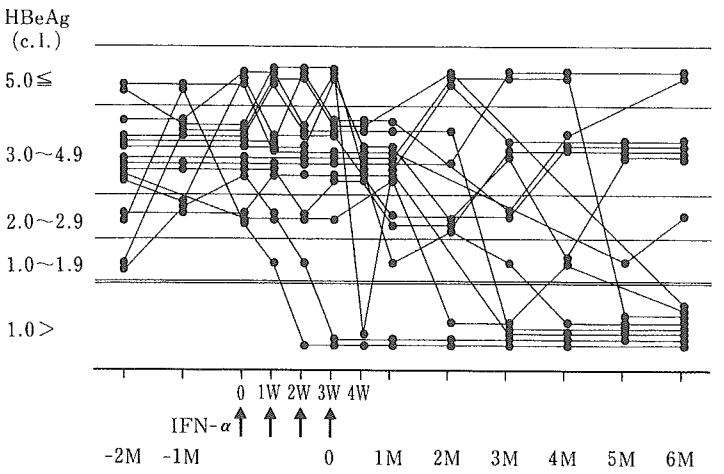

図2　**IFN**少量間欠投与前後の**HBe**抗原カットオフインデックスの変動