Table 1 Comparison of clinical and virological backgrounds between patients with and without relapse of hepatitis at initiation and discontinuation of nucleos(t)ide analogs (NAs)

Background	Non-relapse patients ($n = 32$)	Relapse patients ($n = 53$)	*P*-value
At initiation of NAs			
Age (years)†	47 (17–75)	48 (26–74)	>0.2
Gender (M : F)	23:9	32:21	>0.2
ALT (IU/L)†	183 (9–1182)	187 (20–2052)	>0.2
Genotype (A : B : C : UD)	1:2:21:8	0:3:44:6	0.193
HBeAg (positive)‡	11 (34%)	16 (30%)	>0.2
HBV DNA			
Amplicor assay (log copies/mL)†	6.2 (<2.6–>7.6)	6.5 (<2.6–>7.6)	0.099
HBsAg (log IU/mL)†	2.7 (0.1–4.3)	3.3 (1.6–3.9)	0.018
HBcrAg (log U/mL)†	5.2 (<3.0–>6.8)	5.6 (<3.0–>6.8)	>0.2
At discontinuation of NAs			
Age (years)†	50 (21–78)	49 (26–79)	>0.2
NAs (LVD : LVD+ADV : ETV : ADV)	28:1:3:0	50:0:2:1	>0.2
Duration of NA treatment (months)†	36 (4–129)	17 (4–84)	0.007
Follow-up period after discontinuation of NAs (months)†	45 (6–123)	12 (1–111)	0.002
ALT (IU/L)†	16 (7–38)	20 (9–65)	0.002
HBV DNA			
Amplicor assay (log copies/mL)†	<2.6 (<2.6–2.9)	<2.6 (<2.6–2.9)	>0.2
TaqMan assay (negative signal)‡	5 (23%) ($n = 22$)	3 (14%) ($n = 21$)	>0.2
TaqMan assay (negative or positive signal)‡	13 (59%) ($n = 22$)	13 (62%) ($n = 21$)	>0.2
HBsAg (log IU/ml)†	2.0 (<–1.5–4.3)	3.1 (0.6–4.0)	0.001
HBcrAg (log IU/mL)†	3.4 (<3.0–4.9)	4.3 (<3.0–>6.8)	0.003

†Data are expressed as the median (range)
‡Data are expressed as a positive number (%)
ADV; adefovir dipivoxil; ALT, alanine aminotransferase; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LVD, lamivudine; UD, undetermined.

patients with a negative detection signal or a positive signal also did not vary significantly. The follow-up period after discontinuation of NAs was significantly shorter in patients with relapse than in those without because formal follow-up ended once patients relapsed. The median period of follow-up was 45 months in patients without relapse.

Multivariate analyses revealed that a shorter duration of NA treatment and higher levels of HBsAg and HBcrAg at discontinuation were significantly associated with the occurrence of hepatitis relapse (Table 2). The cut-off values that showed the highest significance by ROC analysis were 1.9 log IU/mL for HBsAg (AUC = 0.707, $P = 0.001$), 4.0 log U/mL for HBcrAg (AUC = 0.692, $P = 0.003$), and 16 months (AUC = 0.674, $P = 0.007$) for treatment duration.

Model for predicting relapse of hepatitis using levels of HBsAg and HBcrAg

The existence of a second cut-off value was suggested by ROC analysis for both of HBsAg (2.9 log IU/mL) and HBcrAg (3.0 log IU/mL) to discriminate between

Table 2 Multivariate analysis of factors associated with relapse of hepatitis after discontinuation of nucleos(t)ide analogs (NAs)

Factor	Hazard ratio	95%CI	*P*-value
HBsAg at discontinuation ≥ 1.9 log IU/mL	5.21	1.87–14.55	0.002
HBcrAg at discontinuation ≥ 4.0 log U/mL	2.20	1.25–3.87	0.006
Duration of NA treatment ≥ 16 months	0.54	0.31–0.93	0.027

CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen.

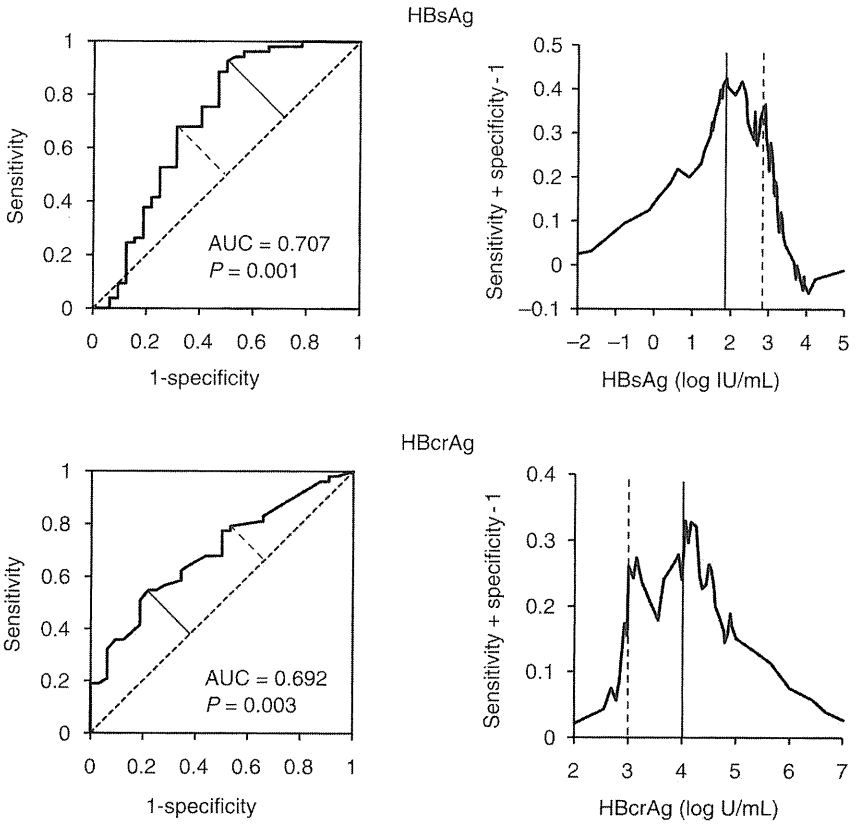


Figure 5 Receiver operating characteristic curve (ROC) analysis of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) to discriminate between patients with and without hepatitis relapse. The existence of two inflection points is suggested for both HBsAg and HBcrAg. Short diagonal lines indicate main inflection points and short broken diagonal lines indicate second inflection points. Vertical lines indicate actual values of antigens that correspond to the main inflection points and vertical broken lines indicate actual values of antigens that correspond to the second inflection points.

patients with and without relapse (Fig. 5). Thus, we set cut-off values as 1.9 and 2.9 log IU/mL for HBsAg and 3.0 and 4.0 log U/mL for HBcrAg in our model for predicting hepatitis relapse.

We tentatively defined three groups using the sum of the scores for HBsAg and HBcrAg levels at the time of NA discontinuation for our model. Conversions were made by assigning a score of 0 for an HBsAg level lower than 1.9 log IU/mL, 1 for a level from 1.9 to 2.8 log IU/mL, and 2 for a level equal to or higher than 2.9 log IU/mL. HBcrAg was scored as 0 for a level lower than 3.0 log U/mL, 1 for a level from 3.0 to 3.9 log U/mL, and 2 for a level equal to or higher than 4.0 log U/mL. Overall, group 1 consisted of patients with a total score of 0, group 2 of patients with a total score of 1 or 2, and group 3 of patients with a total score of 3 or 4.

Patients whose HBV DNA was lower than 3.0 log copies/mL and in whom HBeAg was negative at the time of NA discontinuation were assigned to one of the three groups. Figure 6 shows the comparison of non-relapse rates among the three groups using Kaplan–Meier analysis, which differed significantly. The non-relapse rate was approximately 90% in group 1, as low as 10% in group 3, and intermediate in group 2. When factors associated with relapse were analyzed in group 3 patients, an age of over 40 years at the time of discontinuation was calculated as a significant factor (hazard

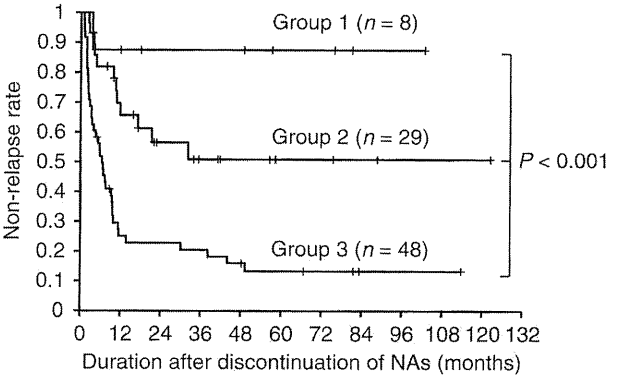


Figure 6 Comparison of non-relapse rates using the Kaplan–Meier method among three groups classified by the sum of the scores of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) levels at the time of nucleos(t)ide analog (NA) discontinuation.

ratio = 5.25, range 2.37–11.65, $P < 0.001$). No significant factors were associated with relapse in group 2 patients.

DISCUSSION

THE EUROPEAN ASSOCIATION for the Study of the Liver recommends continuation of NA treatment until HBsAg is cleared.[25] Liu *et al.* came to a similar conclusion in their study of chronic hepatitis B patients treated with LVD.[14] Indeed, the clearance of HBsAg is a reliable marker for the safe discontinuation of NAs, but the rate of patients who can clear HBsAg is relatively low (1–3%/year).[26–28] Thus, additional factors associated with relapse of hepatitis B after discontinuation of NAs were analyzed in the present study to better identify candidates who could achieve drug-free status. Such studies are relatively few, possibly because patients who discontinue NAs prematurely often experience severe complicating relapse and hepatic failure.[9] Although prospective studies are desirable to obtain accurate results, retrospective studies, such as ours, are also necessary to minimize the risk of adverse complications.

Since HBV cannot be completely eradicated in hosts, the primary goal in treating chronic hepatitis B is to convert symptomatic patients into inactive carriers in whom HBeAg is negative (usually anti-HBe-positive), serum HBV DNA is low, and serum ALT is normal.[1,2,18,29] Thus, we set the clinical conditions of a successful discontinuation of NAs as serum HBV DNA level below 4.0 log copies/mL and ALT below 30 IU/L following NA cessation. Patients who satisfy these conditions are not recommended for treatment by the Japanese guidelines for hepatitis B,[18] and it is also widely accepted that the risk of developing cirrhosis or complicating hepatocellular carcinoma is very low in such patients.[30,31] We used our cohort's mean and maximal values of HBV DNA and ALT for relapse analyses. Mean values were useful for evaluating relapse of hepatitis as a whole since parameter levels often fluctuated after discontinuation, and maximal values were used to evaluate relapse in a real-time fashion during the follow-up period. It is noteworthy that the mean and maximal values correlated very closely for both HBV DNA and ALT. The mean HBV DNA value of 4.0 log copies/mL corresponded to the maximal HBV DNA value of 5.7 by ROC analysis, and similarly the mean ALT value of 30 IU/L corresponded to the maximal ALT value of 79 IU/L. Thus, relapse of hepatitis B was judged to occur when serum ALT became higher than 79 IU/L or when serum HBV DNA surpassed 5.7 log copies/mL after the time of NA discontinuation. Such criteria may also be useful for physicians to detect relapse at an early phase and avoid the occurrence of severe reactivation or unnecessary discontinuation of NAs.

It is generally understood that patients with a higher level of HBV DNA at the time of NA discontinuation are likely to relapse, but this cut-off value has not been analyzed sufficiently. Our findings using ROC analysis showed that patients with levels lower than 3.0 log copies/mL have a good possibility to achieve successful discontinuation. The presence of HBeAg is also generally accepted as a reliable factor to predict relapse of hepatitis. Our study showed that patients with detectable HBeAg at the time of NA discontinuation were likely to relapse, even if their HBV DNA levels were lower than 3.0 log copies/mL. Therefore, we next analyzed additional factors associated with a relapse of hepatitis after discontinuation of NAs by selecting patients who met both of these criteria.

Nucleos(t)ide analog treatment produces a rapid decrease in serum HBV DNA by suppressing reverse transcription of pregenomic HBV RNA. However, the key intrahepatic HBV replicative intermediate, covalently closed circular DNA (cccDNA), tends to remain and is capable of reinitiating replication once NAs are ceased.[32] Measurement of HBV cccDNA has been reported to be useful for monitoring and predicting responses to antiviral treatments.[33] However, its measurement is difficult in the clinical setting as it requires a liver biopsy. Due to the mechanism of action of NAs mentioned above, serum HBV DNA does not reflect intrahepatic HBV cccDNA in patients undergoing NA treatment.[34] To address this, quantitative measurement of HBV antigens has been reported to be useful for predicting the effect of antiviral treatment in patients with chronic hepatitis B. Although HBsAg is usually used as a serum marker for the diagnosis of HBV infection, several groups have shown that HBsAg levels can also be reflective of the response to peg-interferon in chronic hepatitis B.[28,35,36] The HBcrAg assay measures serum levels of HB core and e antigens simultaneously using monoclonal antibodies that recognize the common epitopes of these two denatured antigens. Since the assay measures all antigens transcribed from the pre-core/core gene, it is regarded as core-related.[37] Serum HBcrAg has been reported to accurately reflect intracellular levels of HBV cccDNA even during NA treatment,[24,34,38] and was found to be useful for identifying patients who were likely to show relapse of hepatitis after the discontinuation of NAs.[39,40] It is possible that levels of HBsAg and HBcrAg have different roles in

monitoring antiviral effects because the transcription of these two antigens are regulated by alternative enhancer-promoter systems in the HBV genome.[3] Therefore, we analyzed both of these antigens to elucidate their ability to predict relapse of hepatitis after discontinuation of NAs.

Multivariate analysis demonstrated that levels of HBsAg and HBcrAg at the time of NA discontinuation were independent factors significantly associated with relapse of hepatitis. Thus, we believe these factors can also be applied for predicting relapse in patients whose HBV DNA is lower than 3.0 log copies/mL and whose HBeAg is negative at NA discontinuation. HBV DNA levels were further analyzed using a highly sensitive assay based on real-time polymerase chain reaction (PCR). However, even the level of a negative signal did not ensure successful discontinuation of NAs. The results obtained here indicate that the combined use of HBV-related antigens are useful makers for monitoring the effect of anti-viral treatment in ways different from HBV DNA. Finally, since prolonged NA administration was also a significant factor associated with safe discontinuation, physicians are advised to continue patient treatment for at least 16 months for the best possible outcome.

From our data, a tentative model for predicting relapse of hepatitis after discontinuation of NAs was constructed using levels of HBsAg and HBcrAg at discontinuation. A negative result for HBeAg and HBV DNA lower than 3.0 log copies/mL at the time of NA discontinuation are the essential conditions in this system. Levels of HBsAg and HBcrAg were each converted into scores from 0 to 2 partly because two cut-off values were needed for each antigen and partly because a scoring system may be more convenient for clinical use. The sum of the two scores, which ranged from 0 to 4, was used to prospect relapse. We found that group 1 patients who had a low score (0) could be recommended to discontinue NAs because nearly 90% of this group achieved successful discontinuation. Further analysis of factors associated with relapse are needed for group 2 patients who had middle range scores (1 or 2), since the odds of achieving successful discontinuation were approximately 50%. Continuation of NA treatment is recommended for group 3 patients having high scores (3 or 4) because nearly 90% of this group relapsed. However, this recommendation may be reconsidered in patients younger than 40 years; such cases tended to have a lower relapse rate in group 3. It is also noteworthy that relapse occurred mainly during the first and second years following NA discontinuation in all groups, similarly to a report by Liu *et al.*[14] Thus, clinicians should be vigilant in the early phase after discontinuation.

This study has several limitations. The patients who discontinued NAs were recruited retrospectively, and thus the decision to halt NA treatment was made by individual physicians without uniformly established criteria. Based on this, prospective studies are required to confirm our results. Furthermore, as over 90% of the patients we enrolled had genotype C and over 90% of cases were treated with LVD until discontinuation, the results obtained here can not be applied directly to other HBV genotypes or other types of NAs.

In conclusion, the present study showed that maximal levels of serum ALT and HBV DNA were useful for defining relapse patients after discontinuation of NAs. Along with serum HBV DNA of less than 3.0 log copies/mL and negative serum HBeAg, serum levels of HBsAg and HBcrAg at the time of NA discontinuation were able to predict relapse of hepatitis B and should therefore be considered when establishing uniform guidelines regarding the safe withdrawal of NA treatment. To this end, NA administration of more than 16 months is advisable to achieve successful discontinuation.

ACKNOWLEDGMENTS

THIS RESEARCH WAS supported in part by a research grant from the Ministry of Health, Labor and Welfare of Japan.

We thank Ms. Hiroe Banno for her secretarial assistance and thank Ms. Nozomi Kamijo and Ms. Etsuko Iigahama for their technical assistance. We also thank Mr Trevor Ralph for his English editorial assistance.

REFERENCES

1 Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. *Hepatology* 2007; 45: 1056–75.
2 Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; 45: 507–39.
3 Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997; 337: 1733–45.
4 Ghany M, Liang TJ. Drug targets and molecular mechanisms of drug resistance in chronic hepatitis B. *Gastroenterology* 2007; 132: 1574–85.
5 Liaw YF, Sung JJ, Chow WC *et al.* Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; 351: 1521–31.
6 Matsumoto A, Tanaka E, Rokuhara A *et al.* Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic

hepatitis B: a multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; **32**: 173–84.
7 Suzuki Y, Kumada H, Ikeda K *et al.* Histological changes in liver biopsies after one year of lamivudine treatment in patients with chronic hepatitis B infection. *J Hepatol* 1999; **30**: 743–8.
8 Lok AS, Zoulim F, Locarnini S *et al.* Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. *Hepatology* 2007; **46**: 254–65.
9 Honkoop P, de Man RA, Niesters HG, Zondervan PE, Schalm SW. Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. *Hepatology* 2000; **32**: 635–9.
10 Honkoop P, de Man RA, Heijtink RA, Schalm SW. Hepatitis B reactivation after lamivudine. *Lancet* 1995; **346**: 1156–7.
11 Chang TT, Gish RG, de Man R *et al.* A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001–10.
12 Lai CL, Shouval D, Lok AS *et al.* Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2006; **354**: 1011–20.
13 Lai CL, Chien RN, Leung NW *et al.* A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; **339**: 61–8.
14 Liu F, Wang L, Li XY *et al.* Poor durability of lamivudine effectiveness despite stringent cessation criteria: a prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients. *J Gastroenterol Hepatol* 2011; **26**: 456–60.
15 Reijnders JG, Perquin MJ, Zhang N, Hansen BE, Janssen HL. Nucleos(t)ide analogues only induce temporary hepatitis B e antigen seroconversion in most patients with chronic hepatitis B. *Gastroenterology* 2010; **139**: 491–8.
16 Liaw YF, Leung N, Kao JH *et al.* Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2**: 263–83.
17 Leung N. Recent data on treatment of chronic hepatitis B with nucleos(t)ide analogues. *Hepatol Int* 2008; **2**: 163–78.
18 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40**: 1–7.
19 Schuttler CG, Wend UC, Faupel FM, Lelie PN, Gerlich WH. Antigenic and physicochemical characterization of the 2nd International Standard for hepatitis B virus surface antigen (HBsAg). *J Clin Virol* 2010; **47**: 238–42.
20 Dai CY, Yu ML, Chen SC *et al.* Clinical evaluation of the COBAS Amplicor HBV monitor test for measuring serum HBV DNA and comparison with the Quantiplex branched DNA signal amplification assay in Taiwan. *J Clin Pathol* 2004; **57**: 141–5.
21 Ronsin C, Pillet A, Bali C, Denoyel GA. Evaluation of the COBAS AmpliPrep-total nucleic acid isolation-COBAS TaqMan hepatitis B virus (HBV) quantitative test and comparison to the VERSANT HBV DNA 3.0 assay. *J Clin Microbiol* 2006; **44**: 1390–9.
22 Mizokami M, Nakano T, Orito E *et al.* Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. *FEBS Lett* 1999; **450**: 66–71.
23 Kimura T, Rokuhara A, Sakamoto Y *et al.* Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. *J Clin Microbiol* 2002; **40**: 439–45.
24 Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. *J Med Virol* 2009; **81**: 27–33.
25 European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; **50**: 227–42.
26 Gish RG, Lok AS, Chang TT *et al.* Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. *Gastroenterology* 2007; **133**: 1437–44.
27 Marcellin P. Hepatitis B and hepatitis C in 2009. *Liver Int* 2009; **29** (Suppl 1): 1–8.
28 Moucari R, Lada O, Marcellin P. Chronic hepatitis B: back to the future with HBsAg. *Expert Rev Anti Infect Ther* 2009; **7**: 633–6.
29 Yokosuka O, Kurosaki M, Imazeki F *et al.* Management of hepatitis B: consensus of the Japan Society of Hepatology 2009. *Hepatol Res* 2011; **41**: 1–21.
30 Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130**: 678–86.
31 Chen CJ, Yang HI, Su J *et al.* Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65–73.
32 Werle-Lapostolle B, Bowden S, Locarnini S *et al.* Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology* 2004; **126**: 1750–8.
33 Sung JJ, Wong ML, Bowden S *et al.* Intrahepatic hepatitis B virus covalently closed circular DNA can be a predictor of sustained response to therapy. *Gastroenterology* 2005; **128**: 1890–7.
34 Wong DK, Tanaka Y, Lai CL, Mizokami M, Fung J, Yuen MF. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. *J Clin Microbiol* 2007; **45**: 3942–7.
35 Brunetto MR, Moriconi F, Bonino F *et al.* Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. *Hepatology* 2009; **49**: 1141–50.
36 Moucari R, Mackiewicz V, Lada O *et al.* Early serum HBsAg drop: a strong predictor of sustained virological response

to pegylated interferon alfa-2a in HBeAg-negative patients. *Hepatology* 2009; **49**: 1151–7.
37 Tanaka E, Matsumoto A, Yoshizawa K, Maki N. Hepatitis B core-related antigen assay is useful for monitoring the antiviral effects of nucleoside analogue therapy. *Intervirology* 2008; **51** (Suppl 1): 3–6.
38 Hosaka T, Suzuki F, Kobayashi M *et al.* HBcrAg is a predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy. *Liver Int* 2010; **30**: 1461–70.
39 Matsumoto A, Tanaka E, Minami M *et al.* Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. *Hepatol Res* 2007; **37**: 661–6.
40 Shinkai N, Tanaka Y, Orito E *et al.* Measurement of hepatitis B virus core-related antigen as predicting factor for relapse after cessation of lamivudine therapy for chronic hepatitis B virus infection. *Hepatol Res* 2006; **36**: 272–6.

Hepatology Research 2011; 41: 505–511 doi: 10.1111/j.1872-034X.2011.00807.x

Original Article

Recommendation of lamivudine-to-entecavir switching treatment in chronic hepatitis B responders: Randomized controlled trial

Kentaro Matsuura,[1,2] Yasuhito Tanaka,[1] Atsunori Kusakabe,[2] Shuhei Hige,[5] Jun Inoue,[6] Masashi Komatsu,[7] Tomoyuki Kuramitsu,[8] Katsuharu Hirano,[9] Tomoyoshi Ohno,[3] Izumi Hasegawa,[3] Haruhiko Kobashi,[10] Keisuke Hino,[11] Yoichi Hiasa,[12] Hideyuki Nomura,[13] Fuminaka Sugauchi,[4] Shunsuke Nojiri,[2] Takashi Joh[2] and Masashi Mizokami[14]

[1]*Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences,* [2]*Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences,* [3]*Department of Gastroenterology, Social Insurance Chukyo Hospital,* [4]*Department of Gastroenterology, Nagoya Koseiin Medical Welfare Center, Nagoya,* [5]*Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Sapporo,* [6]*Department of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai,* [7]*Department of Gastroenterology, Akita City Hospital,* [8]*Kuramitsu Clinic, Akita,* [9]*Department of Gastroenterology and Hepatology, Juntendo University Shizuoka Hospital, Izunokuni,* [10]*Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences,* [11]*Department of Hepatology and Pancreatology, Kawasaki Medical University, Okayama,* [12]*Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Ehime,* [13]*The Center for Liver Diseases, Shin-Kokura Hospital, Kitakyushu, and* [14]*The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Japan*

Aim: In the 2007–2008 guidelines of the study group (Ministry of Health, Labor and Welfare of Japan), lamivudine (LAM)-continuous treatment was recommended in patients treated with LAM for more than 3 years who maintained hepatitis B virus (HBV) DNA less than 2.6 log copies/mL, because in these patients LAM resistance might exist and switching treatment to entecavir (ETV) might cause ETV resistance. However, there was no evidence on whether switching treatment to ETV- or LAM-continuous treatment was better in those patients. In the present study, we performed a randomized controlled trial of LAM-to-ETV switching treatment.

Methods: Twenty-seven patients treated with LAM for more than 3 years whose HBV DNA levels were less than 2.6 log copies/mL were enrolled and randomly divided into two groups, LAM-continued group or switching to ETV group. Then, we examined incidence of virological breakthrough (VBT) and breakthrough hepatitis (BTH) in each group.

Results: There was no BTH in any of the patients. VBT was observed in six patients of the LAM group (6/15, 40%), and no patient of the ETV group (0/11, 0%) ($P = 0.02$). The differences of the proportion of cumulated VBT using a log–rank test with Kaplan–Meier analysis were significant between the LAM and ETV groups ($P = 0.025$).

Conclusion: In patients treated with LAM for more than 3 years maintaining HBV DNA less than 2.6 log copies/mL, switching treatment to ETV is recommended at least during the 2 years' follow-up period.

Key words: chronic hepatitis B, entecavir, lamivudine, lamivudine resistance, randomized controlled trial, switching treatment

INTRODUCTION

OVER THE PAST two decades, treatment of chronic hepatitis B (CHB) has greatly improved with the availability of nucleos(t)ide analogs (NA), including lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine, clevudine and tenofovir. NA target

Correspondence: Dr Yasuhito Tanaka, Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuho, Nagoya 467-8601, Japan. Email: ytanaka@med.nagoya-cu.ac.jp
Received 22 January 2011; revision 5 March 2011; accepted 21 March 2011.

the reverse transcriptase of hepatitis B virus (HBV), and are highly effective in suppressing HBV replication and clinical progression to liver cirrhosis and hepatocellular carcinoma in CHB patients.[1–4]

Lamivudine, ADV and ETV are commonly available in Japan. LAM, the first approved NA, has been shown to provide benefit for CHB patients with respect to the reduction of HBV DNA, normalization of alanine aminotransferase (ALT) and improvement of liver histology.[5,6] However, a serious problem of LAM is the high incidence of drug resistance during long-term treatment. The detection rate of LAM resistance has been reported to be 24% at 1 year and 70% after 5 years of treatment.[7–10] Even when the HBV DNA level was maintained at less than 2.6 log copies/mL, the accumulated incidence of LAM resistance reached 65% in patients treated with LAM for a long period (3 to ~10 years).[11] LAM resistance is caused by amino acid substitution(s) at rtM204V/I within the reverse transcriptase domain of the HBV polymerase gene.[12–14] The emergence of a LAM-resistant strain leads to virological breakthrough (VBT) and breakthrough hepatitis (BTH).

Recently, ETV has been demonstrated to exert antiviral efficacy in both NA-naïve and LAM-resistant CHB patients.[15–17] The frequency of ETV resistance has been reported to be 1.2% after 5 years of treatment in NA-naïve CHB patients.[18,19] On the other hand, in switching treatment to ETV for LAM-resistant CHB patients, the cumulative probability of ETV resistance increases.[17,20] After 5 years of treatment, 51% of LAM-refractory patients treated with ETV showed genotypic ETV resistance.[21]

The 2007–2008 guidelines of the study group (Ministry of Health, Labor and Welfare of Japan) for patients on LAM therapy are summarized in Table 1.[22] Regardless of duration of LAM administration, in cases where HBV DNA is more than 2.6 log copies/mL with BTH, ADV add-on treatment was recommended. In patients treated with LAM for less than 3 years who maintained HBV DNA of less than 2.6 log copies/mL or HBV DNA of 2.6 log copies/mL or more without BTH, switching to ETV was recommended. On the other hand, in patients treated with LAM for more than 3 years who maintained HBV DNA of less than 2.6 log copies/mL or HBV DNA of 2.6 log copies/mL or more without BTH, LAM-continuous treatment was recommended because in these patients LAM resistance might exist, and switching treatment to ETV might cause ETV resistance. However, there is insufficient evidence on whether switching treatment to ETV- or LAM-continuous treatment is better for CHB patients treated with LAM for more than 3 years with HBV DNA of less than 2.6 log copies/mL.

In the present study, we performed a randomized controlled trial of LAM-to-ETV switching treatment in CHB patients treated with LAM for more than 3 years who maintained HBV DNA of less than 2.6 log copies/mL.

METHODS

Patients

A TOTAL OF 27 CHB patients (mean age 55 ± 9 years, 17 men) from 11 institutions all over Japan (Hokkaido University Hospital, Tohoku University Hospital, Akita City Hospital, Kuramitsu Clinic, Juntendo University Hospital, Chukyo Hospital, Nagoya City University Hospital, Okayama University Hospital, Kawasaki Medical University Hospital, Ehime University Hospital, Shin-Kokura Hospital) were enrolled from April 2008. All the patients were followed at least 6 months after they were diagnosed with CHB. Their characteristics are shown in Table 2. They were treated with LAM (100 mg/day) for more than 3 years (median 50 months, range 36–106 months). Before starting LAM administration, all patients were positive for hepatitis B surface antigen (HBsAg) in serum, abnormal for ALT, detectable for HBV DNA, and were not

Table 1 2007–2008 guidelines of the study group (Ministry of Health, Labor and Welfare of Japan) for patients on lamivudine treatment

Duration of lamivudine treatment HBV DNA		<3 years	≥3 years
<2.6 log copies/mL, persistently		May be switched to ETV 0.5 mg/day	LAM 100 mg/day
≥2.6 log copies/mL	No BTH†	May be switched to ETV 0.5 mg/day	LAM 100 mg/day
	With BTH	Add on ADV 10 mg/day	Add on ADV 10 mg/day

†After checking for absence of LAM resistance.
ADV, adefovir; BTH, breakthrough hepatitis; ETV, entecavir; HBV, hepatitis B virus; LAM, lamivudine.

Table 2 Characteristics of LAM continuous group and ETV switch group at baseline

	LAM ($n = 15$)	ETV ($n = 11$)	*P*-value
Male	10	6	NS
Age	53 ± 7	57 ± 7	NS
Duration of LAM administration (month)	59 ± 23	55 ± 18	NS
ALT (IU/L)	33 ± 29	28 ± 22	NS
HbeAg positive	1	1	NS

ALT, alanine aminotransferase; ETV, entecavir; HBeAg, hepatitis B e-antigen; LAM, lamivudine; NS, not significant.

infected with hepatitis C virus and HIV. Patients diagnosed with alcoholism, primary biliary cirrhosis or autoimmune hepatitis were excluded.

Study design

The overview of this study design is shown in Figure 1. Twenty-seven patients treated with LAM for more than 3 years were enrolled, who showed HBV DNA of less than 2.6 log copies/mL at entry. They were randomly divided into two groups by each institution, the LAM-continued group (LAM group) or switching to the ETV group (ETV group). The primary end-points were the incidences of VBT and BTH in each group. VBT was defined as having more than 1 log copies/mL increase of HBV DNA level from the nadir on at least two occasions after initial virological response. BTH was defined as showing abnormal ALT level due to LAM or ETV resistance. All subjects were monitored at least every 3-month intervals. At every visit, routine examination with biochemical (ALT, bilirubin, albumin) and virological (HBV DNA level, hepatitis B e-antigen [HBeAg], anti-HBe) assessments took place. The mean follow-up period was 24 ± 3 months.

This study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) on 4 April 2008 as "A randomized trial of lamivudine continuous therapy and entecavir switching therapy for chronic hepatitis B patients treated with lamivudine monotherapy" (no. UMIN000001120).

The study protocol conformed to the Declaration of Helsinki, and was approved by the Committee for Ethics of Medical Experiments on Human Subjects of all the institutions, and written informed consent was obtained from every participant.

Serological and virological markers of HBV

Hepatitis B surface antigen, antibody against HBsAg (anti-HBs), HBeAg and antibody against HBeAg (anti-HBe) were determined using commercially available enzyme immunoassays. HBV DNA was determined by an Amplicor HBV Monitor (Roche Molecular Systems, Branchburg, NJ, USA; detection limit 2.6 log copies/mL)

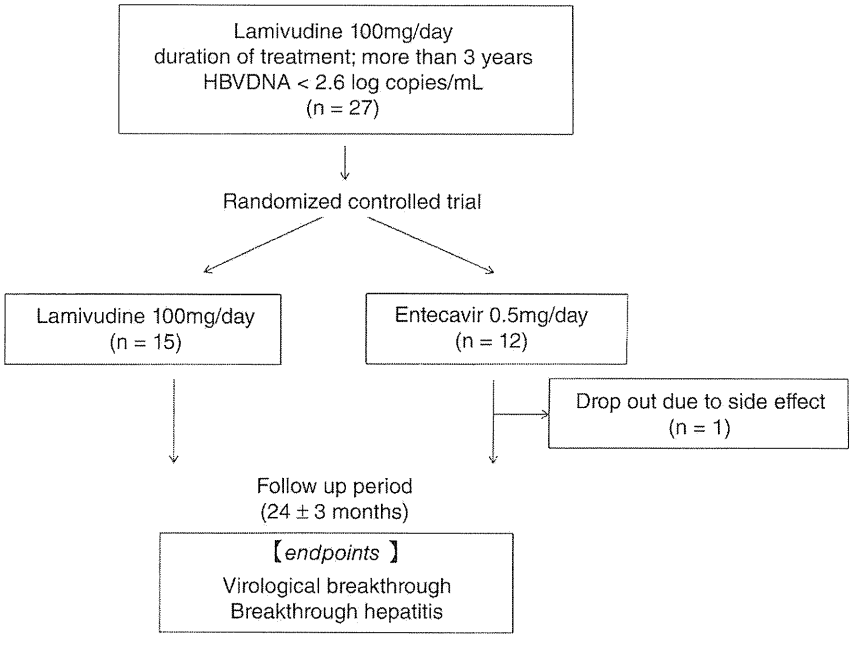


Figure 1 Overview of this study design. Twenty-seven patients treated with lamivudine for more than 3 years whose hepatitis B virus (HBV) DNA was maintained at <2.6 log copies/mL were enrolled. They were randomly divided into two groups by each institution, lamivudine-continued group or switching to entecavir group. We examined the incidence of virological breakthrough and breakthrough hepatitis in each group.

or COBAS AmpliPrep-COBAS TaqMan HBV test (Roche Molecular Systems; detection limit 2.1 log copies/mL). Positive results (signals) below the quantitative HBV DNA concentrations are referred to as "detected" and negative signals are "not detected" when registered by COBAS AmpliPrep-COBAS TaqMan HBV test. The presence of LAM-resistant rtM204V/I and rtL180M substitutions was analyzed by direct sequencing of the HBV DNA polymerase reverse transcriptase site.

Retrospective analysis

Using a conserved serum sample, we examined the existence of LAM-resistant rtM204V/I or rtL180M at baseline in patients with VBT. We also measured HBV DNA by COBAS AmpliPrep-COBAS TaqMan HBV test, and we evaluated the subsequent occurrence of VBT according to the DNA level (not detected/detected/2.1 to <2.6 log copies/mL).

Statistical analysis

Categorial variables were compared between groups by the χ^2-test or Fisher's exact test, and non-categorical variables by Mann–Whitney's *U*-test. The cumulated VBT rate was compared between each group using a log–rank test with Kaplan–Meier analysis. All data were analyzed using SPSS ver. 15.0J software. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of the patients

BASED ON THIS randomized controlled trial, 12 patients were placed in an ETV group and 15 in a LAM group. One patient in the ETV group dropped out because of skin rash by ETV. The baseline characteristics of the patients are described in Table 2. At the entry, one patient was positive for HBeAg in each group. There was no difference in sex, age, duration of LAM administration and ALT level between the two groups.

Incidence of VBT and BTH

There was no BTH in any of the patients. The incidence of VBT was six patients out of 15 (40%) in the LAM group, and no patient in the ETV group ($P = 0.02$). The Kaplan–Meier curve for the proportion of cumulated VBT is shown in Figure 2. The differences in the rates of VBT were significant between the LAM and ETV groups (log–rank test $P = 0.025$).

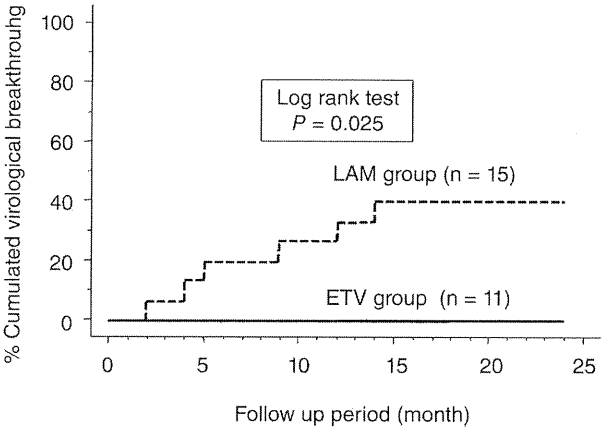


Figure 2 Proportion of cumulated virological breakthrough in lamivudine (LAM) and entecavir (ETV) group. The cumulated rate of virological breakthrough was higher in patients treated with LAM than those with ETV (40% vs 0%, $P = 0.025$ by log–rank test).

Characteristics of patients with VBT in LAM group

Details of the six VBT cases in the LAM group are described in Table 3. Assessment of LAM-resistant mutations at the time of VBT showed that both rtM204V and rtL180M were observed in all cases. For five of the six cases, HBV DNA was detected by COBAS AmpliPrep-COBAS TaqMan HBV test at baseline, although the HBV DNA level was very low. With respect to LAM-resistant mutation at baseline, rtM204V and rtL180M were observed in one of six cases. In contrast, no LAM-resistant mutations were observed in 20 non-VBT cases at baseline.

Incidence of VBT based on the HBV DNA level by COBAS AmpliPrep-COBAS TaqMan HBV test

Incidence of VBT based on the HBV DNA level according to COBAS AmpliPrep-COBAS TaqMan HBV test at baseline is shown in Figure 3. HBV DNA levels were less than 2.6 log copies/mL by Amplicor HBV Monitor in all cases. However, HBV DNA levels in the LAM group were "not detected" in five cases, "detected" in eight cases and 2.1 log copies/mL or more in two cases by COBAS AmpliPrep-COBAS TaqMan HBV test. VBT was observed in five of the 10 cases whose results were either "detected" or 2.1 log copies/mL or more and in one of the five "not detected" cases. On the other hand, although HBV DNA levels in the ETV group were

Table 3 Characteristics of patients with virological breakthrough in LAM group

		At baseline				At virological breakthrough		
Age	Sex	Duration of LAM administration (month)	HBeAg	HBV DNA by TaqMan HBV (log copies/mL)	Mutant of LAM resistance	Period of VBT (months)	HBV DNA (log copies/mL)	Mutant of LAM resistance
49	M	37	Negative	Detected	None	14	4.9	L180M/M204V
54	F	106	Negative	Detected	None	5	2.8	L180M/M204V
63	F	81	Negative	Not detected	None	9	4.5	L180M/M204V
57	F	43	Negative	Detected	None	10	3	L180M/M204V
55	M	84	Negative	Detected	None	12	2.8	L180M/M204V
57	M	36	Negative	2.3	L180M/M204V	2	4	L180M/M204V

ALT, alanine aminotransferase; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; LAM, lamivudine; VBT, virological breakthrough.

"detected" in six cases by COBAS AmpliPrep-COBAS TaqMan HBV test, there was no incidence of VBT: HBV DNA levels of five patients were undetectable and that of one patient was "detected" at the last follow-up point after switching to ETV.

DISCUSSION

AT PRESENT, LAM, ADV and ETV are only approved for treatment of CHB patients in Japan. ETV has become the first-line treatment for NA-naïve patients, because the ETV resistance is much less frequent than LAM-resistance.[8,23,24] On the other hand, in switching treatment to ETV for LAM-resistant CHB patients, the frequency of ETV resistance was increased.[17,20,25–27] It has also been reported that ADV add-on treatment suppressed HBV replication more effectively than ETV or ADV monotherapy in patients with LAM-resistant CHB.[25,28] Therefore, it is desirable to examine LAM-resistant mutants before switching to ETV in patients treated with LAM. However, as the assay for the LAM-resistant mutants is not covered by the Japanese health insurance system at present, the Japanese guidelines for CHB management after LAM therapy were based on HBV DNA, duration of LAM administration and incidence of BTH (Table 1).[22] In patients treated with LAM for more than 3 years, maintaining HBV DNA of less than 2.6 log copies/mL or HBV DNA of 2.6 log copies/mL or more without BTH, LAM-continuous treatment was recommended because in these patients, LAM-resistance might exist, and switching treatment to ETV might cause ETV-resistance. It was reported that although LAM-resistant strains were detected in 34% cases treated with LAM for more than 3 years and whose HBV DNA level was suppressed to less than 2.6 log copies/mL, switching to ETV maintained undetectable HBV DNA level over 2 years.[29] In addition, Kurashige *et al.* reported that LAM-to-ETV switching treatment maintained an undetectable HBV DNA level in patients with baseline HBV DNA of less than 2.6 and 2.6 to less than 4.0 log copies/mL for a period of ETV treatment ranging 10–23 (median 20) months.[30] In the present study, randomized controlled trial evidenced that switching treatment to ETV or LAM-continuous treatment would be recommended in CHB patients treated with LAM for more than 3 years and maintained HBV DNA of less than 2.6 log copies/mL. Interestingly, even though HBV DNA had been suppressed to less than 2.6 log copies/mL, a high rate of VBT was observed in the LAM group, whereas no VBT over 24 months was observed in the ETV group. Of the six patients with VBT,

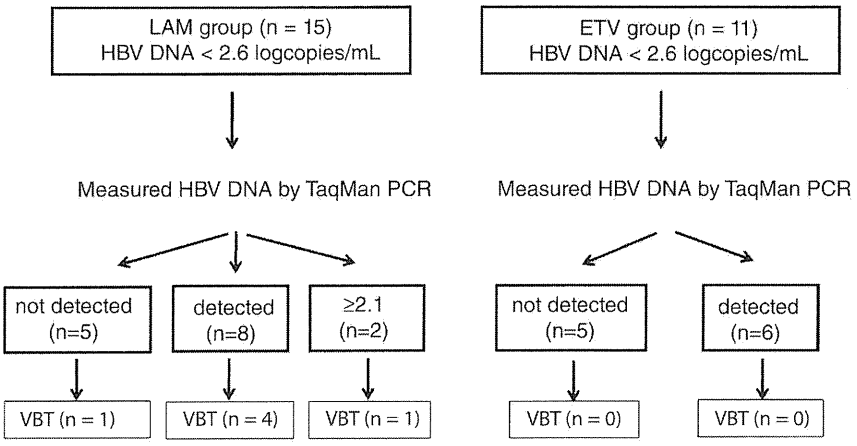


Figure 3 Incidence of virological breakthrough (VBT) based on the hepatitis B virus (HBV) DNA level at baseline by COBAS AmpliPrep-COBAS TaqMan HBV test (TaqMan PCR). The subsequent occurrence of VBT according to the DNA level by TaqMan PCR (not detected/detected/2.1 to <2.6 log copies/mL) was evaluated. In the lamivudine (LAM) group, VBT was observed in five of the 10 cases in which the results were either "detected" or ≥2.1 log copies/mL and in one of the five "not detected" cases. On the other hand, HBV DNA levels in the entecavir (ETV) group were "detected" in six, but there was no incidence of VBT.

five had no LAM resistance at baseline. However, the LAM resistance of rtM204V and rtL180M were found in all the patients with VBT in the LAM group. Moreover, a retrospective assessment by COBAS AmpliPrep-COBAS TaqMan HBV test showed that HBV DNA was detectable in 10 patients in the LAM group and six patients in the ETV group. Only five of the 10 patients in the LAM group had VBT, but none in the ETV group. In addition, one patient had VBT in the LAM group even though DNA was not detected by the TaqMan test, suggesting that switching to ETV was preferable. Hence, our data supported the 2010 Japanese guidelines which recommend switching to ETV in patients whose HBV DNA levels are less than 2.1 log copies/mL by TaqMan PCR.

A potential limitation of the present study is that the number of the cases was small. Nevertheless, our randomized controlled trial indicated significant difference in the incidence of VBT between the LAM and ETV groups. Therefore, this study is valuable for the purpose of verifying the 2007–2008 guidelines in Japan. In the present study, although no LAM-resistant mutant was observed in the ETV group at baseline, a very low level of LAM-resistant mutants may derive ETV resistance for long-term therapy. The results of switching to ETV in the present study were favorable during the 24-month observation period, but we have to be careful of possible emergence of ETV-resistant mutants in long-term follow up.

In conclusion, in patients treated with LAM for more than 3 years maintaining HBV DNA of less than 2.6 log copies/mL, switching treatment to ETV is recommended in at least a 2-year follow-up period.

ACKNOWLEDGMENTS

WE WOULD LIKE to thank Yoshiyuki Ueno (Tohoku University), Takafumi Ichida (Juntendo University), Dr Moriichi Onji (Ehime University), Dr Kazuhide Yamamoto (Okayama University) and Dr Masaaki Korenaga (Kawasaki Medical School) for their advice throughout the study. The study was supported in part by a grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology.

REFERENCES

1 Liaw YF, Sung JJ, Chow WC *et al.* Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; **351**: 1521–31.
2 Liaw YF. Hepatitis B virus replication and liver disease progression: the impact of antiviral therapy. *Antivir Ther* 2006; **11**: 669–79.
3 Di Marco V, Marzano A, Lampertico P *et al.* Clinical outcome of HBeAg-negative chronic hepatitis B in relation to virological response to lamivudine. *Hepatology* 2004; **40**: 883–91.
4 Papatheodoridis GV, Dimou E, Dimakopoulos K *et al.* Outcome of hepatitis B e antigen-negative chronic hepatitis B on long-term nucleos(t)ide analog therapy starting with lamivudine. *Hepatology* 2005; **42**: 121–9.

5 Lai CL, Chien RN, Leung NW *et al.* A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; **339**: 61–8.
6 Dienstag JL, Schiff ER, Wright TL *et al.* Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 1999; **21** (341): 1256–63.
7 Lai CL, Dienstag J, Schiff E *et al.* Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. *Clin Infect Dis* 2003; **36**: 687–96.
8 Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661–2.
9 Lok AS, Lai CL, Leung N *et al.* Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003; **125**: 1714–22.
10 Chang TT, Lai CL, Chien RN *et al.* Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. *J Gastroenterol Hepatol* 2004; **19**: 1276–82.
11 Kobayashi M, Suzuki F, Akuta N *et al.* Correlation of YMDD mutation and breakthrough hepatitis with hepatitis B virus DNA and serum ALT during lamivudine treatment. *Hepatol Res* 2010; **40**: 125–34.
12 Allen MI, Deslauriers M, Andrews CW *et al.* Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. *Hepatology* 1998; **27**: 1670–7.
13 Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. *Hepatology* 1999; **30**: 567–72.
14 Westland CE, Yang H, Delaney W *et al.* Activity of adefovir dipivoxil against all patterns of lamivudine-resistant hepatitis B viruses in patients. *J Viral Hepat* 2005; **12**: 67–73.
15 Chang TT, Gish RG, de Man R *et al.* A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001–10.
16 Lai CL, Shouval D, Lok AS *et al.* Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2006; **354**: 1011–20.
17 Sherman M, Yurdaydin C, Sollano J *et al.* Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. *Gastroenterology* 2006; **130**: 2039–49.
18 Colonno RJ, Rose R, Baldick CJ *et al.* Entecavir resistance is rare in nucleoside naive patients with hepatitis B. *Hepatology* 2006; **44**: 1656–65.
19 Tenney DJ, Rose RE, Baldick CJ *et al.* Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naive patients is rare through 5 years of therapy. *Hepatology* 2009; **49**: 1503–14.
20 Sherman M, Yurdaydin C, Simsek H *et al.* Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical, and serology outcomes through 96 weeks. *Hepatology* 2008; **48**: 99–108.
21 Tenney DJ, Pokornowski K, Rose RE *et al.* Entecavir at five years shows long-term maintenance of high genetic barrier to hepatitis B virus resistance. *Hepatol Int* 2008; **2**: S302–3.
22 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40**: 1–7.
23 Liaw YF, Leung N, Kao JH *et al.* Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2**: 263–83.
24 European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; **50**: 227–42.
25 Reijnders JG, Deterding K, Petersen J *et al.* Antiviral effect of entecavir in chronic hepatitis B: influence of prior exposure to nucleos(t)ide analogues. *J Hepatol* 2010; **52**: 493–500.
26 Suzuki Y, Suzuki F, Kawamura Y *et al.* Efficacy of entecavir treatment for lamivudine-resistant hepatitis B over 3 years: histological improvement or entecavir resistance? *J Gastroenterol Hepatol* 2009; **24**: 429–35.
27 Mukaide M, Tanaka Y, Shin IT *et al.* Mechanism of entecavir resistance of hepatitis B virus with viral breakthrough as determined by long-term clinical assessment and molecular docking simulation. *Antimicrob Agents Chemother* 2010; **54**: 882–9.
28 Kim HJ, Park JH, Park DI *et al.* Rescue therapy for lamivudine-resistant chronic hepatitis B: comparison between entecavir 1.0 mg monotherapy, adefovir monotherapy and adefovir add-on lamivudine combination therapy. *J Gastroenterol Hepatol* 2010; **25**: 1374–80.
29 Suzuki F, Akuta N, Suzuki Y *et al.* Efficacy of switching to entecavir monotherapy in Japanese lamivudine-pretreated patients. *J Gastroenterol Hepatol* 2010; **25**: 892–8.
30 Kurashige N, Ohkawa K, Hiramatsu N *et al.* Lamivudine-to-entecavir switching treatment in type B chronic hepatitis patients without evidence of lamivudine resistance. *J Gastroenterol* 2009; **44**: 864–70.

特集II　B型肝炎に対する新治療戦略

Entecavirによる抗ウイルス療法*

小関　至**
狩野吉康**
豊田成司**

Key Words：lamivudine, adefovir, entecavir, hepatitis B virus, resistance mutation

はじめに

Lamivudine(LAM)，adefovir dipivoxil(ADV)に続く3番目の核酸アナログ製剤として2006年に承認されたentecavir(ETV)は，既存の核酸アナログ製剤と比較して高い抗ウイルス効果を示し，さらには，耐性変異株の出現がきわめて低率であること[1)~3)]，また，ADVのような腎機能障害[4)5)]を認めないことから，現在，本邦の核酸アナログ製剤の中では第一選択薬剤となった．2011年度のB型慢性肝炎の治療ガイドラインにおいても，35歳以上の中高年に対しては第一選択薬剤とされた．

一方で，中止を考慮する際，肝炎再燃のリスクの予測が困難であること，生殖年齢に対する，特に挙児希望例に対する投与は実臨床では困難であること，ならびに低率であるがETV耐性が出現してしまった症例に対する十分な抗ウイルス活性を有する薬剤が本邦では存在しないこと，human immunodeficiency virus(HIV)合併例では禁忌であることなどが問題点としてあげられる．

本稿では当院におけるETVの抗ウイルス効果，hepatitis B virus(HBV)関連マーカーの推移，肝予備能改善効果，耐性出現率について基説する．

対象と方法

2011年4月までに当院においてETVを初回投与した314例中，少なくとも1年以上経過を追えた204例を対象とした．自己免疫性肝炎，アルコール性肝障害，うっ血性肝障害の併発例，C型肝炎ウイルスあるいはHIV合併例，HBV再活性化予防目的のETV投与例，過去に核酸アナログ製剤投与を受けた症例は除外した．

204例の背景因子の内訳を表1に示す．患者因子では年齢中央値は56歳，治療期間中央値31か月，男性137例(67.2%)，肝硬変例63例(30.1%)，interferon(IFN)投与歴あり20例(9.8%)，hepato-

表1　患者背景因子

年齢，歳	56(23~82)	HBeAg陽性	69(33.8%)
治療期間，月	31(13~55)	HBsAg, log IU/ml	3.2(−0.5~5.8)
性，男性	137(67.2%)	crAg, log U/ml	5.4(3.0~6.8)
肝病変，肝硬変	63(30.1%)	HBV DNA, log copies/ml	6.7(2.1~9.0)
IFN治療歴，あり	20(9.8%)	ALT, IU/l	77(17~1830)
HCC治療，あり	52(25.5%)	Genotype, A/B/C/H/ND	5/28/151/1/19

* Antiviral effect of entecavir.

** Itaru OZEKI, M.D., Yoshiyasu KARINO, M.D. & Joji TOYOTA, M.D.: 札幌厚生病院第3消化器科〔〒060-0033 札幌市中央区北3条東8丁目5〕; Department of Hepatology, Sapporo Kousei General Hospital, Sapporo 060-0033, JAPAN

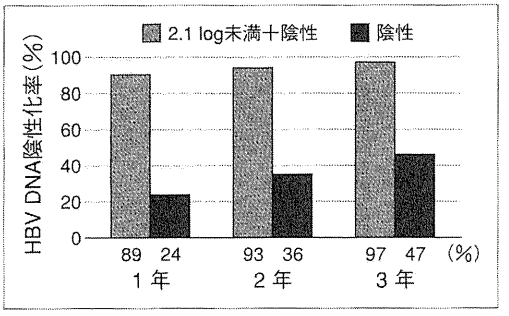


図1　HBV DNA陰性化率
TaqMan PCR法によるHBV DNA陰性化率の推移を示す．2.1 log未満と陰性例を加えた症例の割合は1年で89%，2年で93%，3年で97%，陰性例だけを抽出すると1年24%，2年36%，3年47%であった．

cellular carcinoma(HCC)治療歴あり52例(25.5%)であった．ウイルス側因子ではHBe抗原陽性69例(33.8%)，HBs抗原中央値3.2 logIU/m*l*(以下単位略)，コア関連抗原中央値5.4 logU/m*l*(以下単位略)，HBV DNA中央値 6.7 logcopies/m*l*(以下単位略)，ALT中央値77 IU/*l*，遺伝子型A型，B型，C型，H型がそれぞれ5例(2.7%)，28例(15.1%)，151例(81.6%)，1例(0.5%)であった．

HBV DNAはTaqMan PCR法(Roche Diagnostics, Tokyo, Japan)，遺伝子型はPCR-Invader法(BML, Tokyo, Japan)，HBs抗原はCLIA法，コア関連抗原はCLEIA法，耐性部位の検討はINNO-LiPA HBV DR version 2, version 3(Innogenetics Gent, Belgium)を用いた．なお，HBs抗原量は実数から対数に変換し検討を行った．

検討項目として①HBV DNA陰性化率，②ALT異常例の検討，③HBs抗原とコア関連抗原の推移，④肝予備能改善効果，⑤ETV耐性出現率を検討した．③の検討ではETV3年以上投与可能であった68例，④の検討ではETV3年以上投与可能で，かつ，HBs抗原とコア関連抗原が経時的に測定できた61例を対象とした．

抗ウイルス効果の検討

1．HBV DNA陰性化率

TaqMan PCR法にて測定した1年，2年，3年の3 pointsの成績を示す．陰性化率は2.1 log未満と陰性例を合わせると，1年89%，2年93%，3年97%，陰性例のみに限ると1年24%，2年36%，3年47%を示した(図1)．HBV DNA 2.6 log未満を陰性と定義した時の当院のLAM 1年以上投与例(187例)のHBV DNA陰性化率が1年64%，2年57%，3年44%であった．LAM投与時では，耐性株出現のためHBV DNA陰性化率は経年的に低くなるが，LAMと比較してもETVの優れた抗ウイルス効果は明らかである．

ETV投与2年時においてHBV DNAがTaqMan PCR法で陰性あるいは2.1未満を示した症例を陰性と定義すると，HBV DNA陰性例は127例，2.1 log以上のHBV DNA陽性例は13例であった．13例のうち，多剤耐性出現1例と明らかにコンプライアンスが不良であった1例を除く11例で解

表2　HBV DNA陽性例と陰性例の背景因子の比較

	陰性 n＝127	陽性 n＝11	*P*
年齢	55(23～78)	44(28～72)	0.089
男性	85(69)	8(73)	0.954
肝硬変	41(33)	3(27)	0.996
e抗原陽性	43(35)	5(46)	0.439
ALT	83(17～1478)	68(33～294)	0.877
HBV DNA	6.6(2.6～8.8)	7.6(4.6～8.8)	0.028
HBsAg	3.2(0.5～5.8)	3.9(1.2～5.0)	0.068
crAg	5.5(3.0～6.8)	5.7(3.8～6.8)	0.743
Genotype C	92/116(79)	7/10(70)	0.774

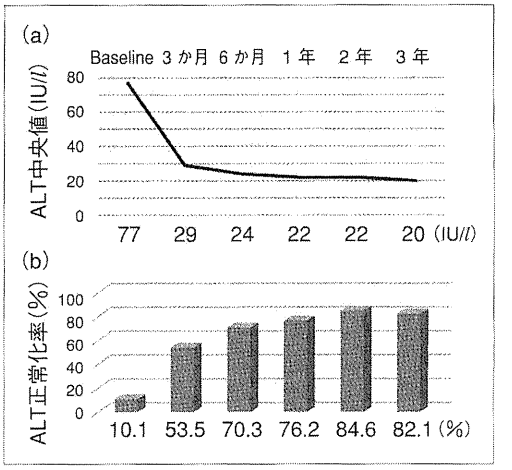


図2　ALT正常化率
(a)ALT中央値は治療開始時，3か月後，6か月後，1年後，2年後，3年後で，それぞれ，77 IU/*l*，29 IU/*l*，24 IU/*l*，22 IU/*l*，22 IU/*l*，20 IU/*l*であった．(b)ALT30 IU/*l*以下を正常として正常化率を算出した．治療開始時，3か月後，6か月後，1年後，2年後，3年後で，それぞれ，10.1%，53.5%，70.3%，76.2%，84.6%，82.1%であった．

表 3-a ALT正常例と異常例の比較

	正常 n＝111	異常 n＝29	*P*
年齢	55(26～78)	56(23～73)	0.902
男性	77(69)	20(69)	0.967
肝硬変	35(32)	10(35)	0.762
HCC合併あり	23(21)	12(41)	0.022
BMI 25以上	24(22)	16(55)	0.009
高血圧	21(19)	7(24)	0.532
糖尿病	19(17)	5(17)	0.987
高脂血症	19(17)	9(31)	0.095
脂肪肝	14(13)	6(21)	0.267
ALT	83(17～1487)	73(18～774)	0.765
HBV DNA	6.7(2.1～8.8)	6.6(2.6～8.8)	0.339
HBsAg	3.2(0.5～5.8)	3.2(0.9～5.0)	0.909
crAg	5.7(3.0～6.8)	5.2(3.0～6.8)	0.359
Genotype C	81(76)	23(96)	0.054
e 抗原陽性	40(36)	9(31)	0.615
ETV 2 年時DNA≧2.1	6(5)	4(14)	0.247

表 3-b ALT異常に寄与する因子－多変量解析－

因子	カテゴリー	オッズ比(95％信頼区間)	*P* 値
HCC合併	1. No	1	0.040
	2. Yes	2.617(1.047～6.538)	
BMI	1. 25未満	1	0.001
	2. 25以上	4.411(1.830～10.635)	

析を行った．HBV DNA陽性例は陰性例と比較するとbaselineのHBV DNA量が有意に高値を示した(表 2)．

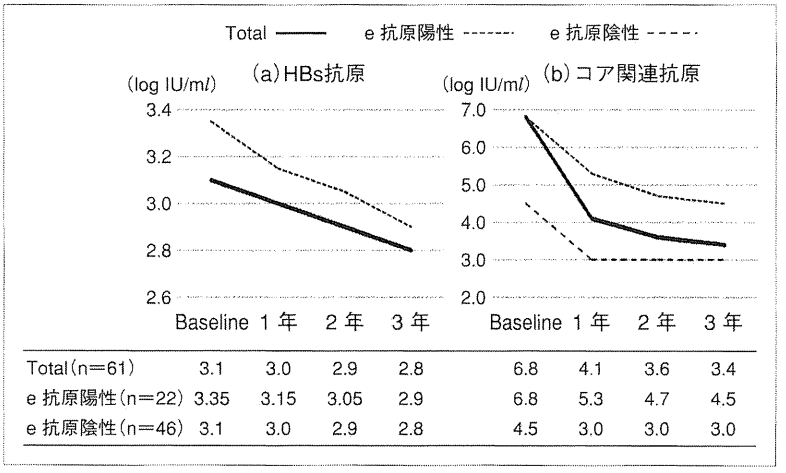
2．ALTの推移と陰性化率

ALT中央値は治療開始時，3 か月後，6 か月後，1 年後，2 年後，3 年後で，それぞれ，77 IU/*l*，29 IU/*l*，24 IU/*l*，22 IU/*l*，22 IU/*l*，20 IU/*l*であった．ALT30 IU/*l*以下を正常として正常化率を算出すると，それぞれ，10.1％，53.5％，70.3％，76.2％，84.6％，82.1％であった(図 2)．ETV投与 2 年の時点におけるALT異常例と正常例を背景因子にETV投与 2 年時のHBV DNA量の治療後因子を加えて比較すると，HCC合併例とBMI 25以上の割合が異常例で有意に高率を示した(表 3-a)．多変量解析にてHCC合併はオッズ比2.6倍，BMI 25以上はオッズ比4.4倍でALT異常に寄与する因子として抽出された(表 3-b)．すなわち，ETV投与を行い，良好なウイルスコントロールが可能であってもALTが異常を示す症例では，HCCの治療中の症例，あるいは肥満を有する症例の可能性が高いと考えられた．

3．HBs抗原とコア関連抗原の推移

ETVは前述のように投与数年後にはほとんどの症例で血中のHBV DNAが陰性化するので，肝細胞内に残存するHBVウイルス(ccc DNA)量と乖離する現象が生じる．ETV投与例における抗ウイルス効果の類推やその症例の予後を推測する上で，また，ETV投与中止を判断する指標としてもHBs抗原量とコア関連抗原が期待されているが，これらの検査値の臨床的意義は不明な点も多い．

3 年以上ETVを投与した68例中，HBs抗原とコア関連抗原の経時的推移を観察できた61例(e 抗原陽性22例，陰性39例)を対象とした．HBs抗原はTotal61例ではbaseline 3.1 log，1 年3.0 log，2 年2.9 log，3 年2.8 logと緩徐に低下した．e 抗原陽性例ではbaseline 3.35 log，1 年3.15 log，2 年3.05 log，3 年2.9 log，e 抗原陰性例はbaseline 3.1 log，1 年3.0 log，2 年2.9 log，3 年2.8 logであった．e 抗原の有無にかかわらず，HBs抗原の減衰は緩徐であった．一方で，コア関連抗原はbaseline 6.8 log，1 年4.1 log，2 年3.6 log，3 年3.4 log，e



	(a)HBs抗原				(b)コア関連抗原			
	Baseline	1年	2年	3年	Baseline	1年	2年	3年
Total(n=61)	3.1	3.0	2.9	2.8	6.8	4.1	3.6	3.4
e抗原陽性(n=22)	3.35	3.15	3.05	2.9	6.8	5.3	4.7	4.5
e抗原陰性(n=46)	3.1	3.0	2.9	2.8	4.5	3.0	3.0	3.0

図3　HBs抗原とコア関連抗原の推移

(a)HBs抗原はbaseline 3.1 log, 1年3.0 log, 2年2.9 log, 3年2.8 logと緩徐に低下した．e抗原の有無別の推移では陽性例ではbaseline 3.35 log, 1年3.15 log, 2年3.05 log, 3年2.9 log, 陰性例はbaseline 3.1 log, 1年3.0 log, 2年2.9 log, 3年2.8 logであった(Totalとe抗原陰性は同じ値を示した)．(b)コア関連抗原はbaseline 6.8 log, 1年4.1 log, 2年3.6 log, 3年3.4 log, e抗原陽性例では同様に6.8 log, 5.3 log, 4.7 log, 4.5 log, e抗原陰性例では4.5 log, 3.0 log, 3.0 log, 3.0 logを示した．

アルブミン

	開始	1年	2年	3年
Total	3.9	4.1	4.3	4.2
慢性肝炎	3.9	4.1	4.3	4.3
肝硬変	3.9	3.9	4.2	4.0

血小板

	開始	1年	2年	3年
Total	14.8	16.3	15.0	14.8
慢性肝炎	15.8	17.1	16.5	15.8
肝硬変	10.1	9.2	9.3	8.5

プロトロンビン時間

	開始	1年	2年	3年
Total	86	97	102	105
慢性肝炎	90	99	103	107
肝硬変	71	86	87	88

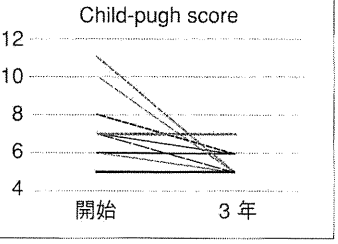


図4　肝予備能改善効果

アルブミンとプロトロンビン時間は背景肝病変が慢性肝炎，肝硬変にかかわらず投与3年の経過で改善を，血小板数はプラトーを示した．肝硬変例のChild-pugh scoreの推移をみると，8例でscore変化なし，9例でscoreの改善が得られた．

抗原陽性例では同様に6.8 log, 5.3 log, 4.7 log, 4.5 log, e抗原陰性例では4.5 log, 3.0 log, 3.0 log, 3.0 logであった．コア関連抗原はbaselineの時点でe抗原陽性例と陰性例ですでに2 log以上の乖離が認められた．また，e抗原陰性例では測定限界値である3 logを示す症例が多くなった．コア関連抗原の推移をみる場合は，e抗原陽性例と陰性例を分けて検討する必要があると考えられる(図3)．

4．肝予備能改善効果

3年以上ETVを投与できた68例(慢性肝炎51例，肝硬変17例)で，アルブミン，血小板，プロトロンビン時間の経時的推移を算出し，さらに肝硬変17例に対してChild-pugh scoreをETV開始時と

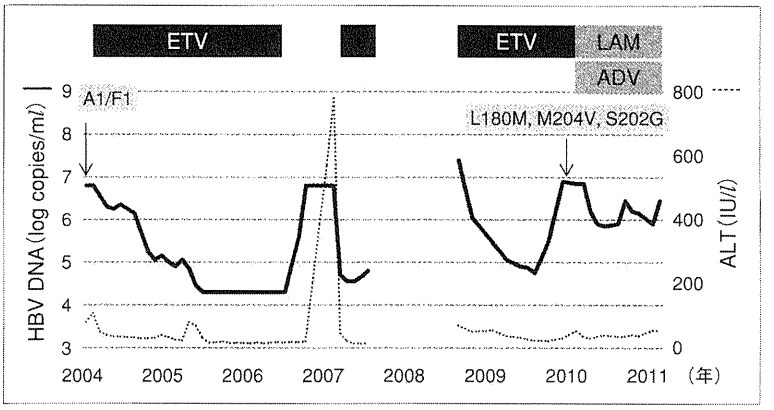


図5　ETV耐性が出現した1例
観察期間中央値31か月の経過でETV初回投与204例中1例(0.5%)でETV耐性(L180M, M204V, S202G)が出現した.

投与3年時で比較した.

アルブミンとプロトロンビン時間は背景肝病変が慢性肝炎，肝硬変にかかわらず投与3年の経過で改善を示した．一方，血小板は慢性肝炎ではプラトー，肝硬変ではわずかに低下した．肝硬変例のChild-pugh scoreの推移では，8例でscore変化なし，9例でscoreの低下をきたした(図4)．このようにETV投与例では明らかに肝予備能の改善が得られた．

5．ETV耐性出現率

観察期間中央値31か月の経過で204例中1例(0.5%)でETVのS202Gの変異が出現した(図5)．この症例は38歳男性，e抗原陽性で，遺伝子型はC型であった．2004年6月にETVの容量比較試験に参加し，ETVが開始された．なお，試験参加直前の肝生検では新犬山分類でA1F1であった．2006年9月試験終了に伴い，ETVが中止されるも，肝炎の再燃のため，2007年4月ETV再開．同年10月より受診されず，2008年10月に約1年の期間を空けて来院．HBV DNA 8.8 log, ALT70 IU/lにてETV再開も，2010年1月にviral breakthroughをきたし，前述のETV耐性が確認された．2010年4月からlamivudineとadefovir併用を行うも，HBV DNAは6 log前後で推移している．この症例は2回にわたりETVを中断したという特殊な経緯を有していた．この1例を除くと，現在，ETV初回投与例からのETV耐性例は出現していない．

おわりに

ETVは抗ウイルス作用が強力で耐性出現はきわめて稀であり，初回治療例では第一選択の薬剤である．今後は長期投与による肝発癌抑止率を明らかにするとともに，HBs抗原消失すなわち臨床的治癒例の詳細な検討が必要である．

文　献

1) Sherman M, Yurdaydin C, Sollano J, et al. Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. Gastroenterology 2006 ; 130 : 2039.
2) Lai CL, Shouval D, Lok AS, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006 ; 354 : 1011.
3) Tenney DJ, Rose RE, Baldick CJ, et al. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. Hepatology 2009 ; 49 : 1503.
4) Marcellin P, Chang TT, Lim SG, et al. Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. Hepatology 2008 ; 48 : 750.
5) Ha NB, Ha NB, Garcia RT, et al. Renal dysfunction in chronic hepatitis B patients treated with adefovir dipivoxil. Hepatology 2009 ; 50 : 727.

B型肝炎

B型肝炎の抗ウイルス療法の実際

狩野吉康・小関　至・豊田成司

札幌厚生病院第三消化器科／かりの・よしやす・おぜき・いたる・とよた・じょうじ

はじめに●

わが国のB型肝炎の抗ウイルス療法は1986年にインターフェロン(IFN)βが保険適応となった以降はインターフェロン治療が主役であった．2000年にラミブジンが保険適応になってからは副作用の少ない経口薬であるという側面もあり，核酸アナログ製剤が抗ウイルス療法の中心となっている．当面の課題は作用機序の異なる二つの薬剤をいかに有効に用いて治療効果を向上させるかである．本稿ではわが国のB型肝炎の抗ウイルス療法の実際について解説する．

B型肝炎に対する抗ウイルス療法の適応と治療目標●

B型慢性肝炎の治療目標はB型肝炎ウイルス(HBV)の持続的な増殖抑制であり，これが達成されると肝炎の鎮静化，肝予備能の改善が得られ，さらには肝発癌の抑制も期待される．

2009年度の厚生労働省班会議の「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」の結果を基にした治療ガイドラインでは，抗ウイルス療法の対象をALT 31 IU/l以上としている．治療目標は最終的にはHBs抗原陰性化を目指し，35歳未満ではdrug freeを念頭にIFN単独治療あるいは核酸アナログ・IFNのsequential療法を，35歳以上ではHBV-DNAの持続的陰性化およびALT値の持続正常化を目指して核酸アナログ製剤長期投与を推奨している．治療導入のタイミングとしてはF2以上の組織進展例においては可及的速やかに抗ウイルス療法開始が望まれる．肝組織が進展していない若年者においては，短期間の肝炎期を経て，HBe抗原・抗体のセロコンバーション(SC)の後，肝炎の鎮静化が得られる場合も多いため，経過観察した上で，抗ウイルス療法導入の可否を決定するべきである．またB型肝炎ではHBV genotypeにより自然予後が異なることが明らかになっている，すなわちわが国のB型肝炎の大部分を占めるgenotype BとCではgenotype Bで自然経過中にSCが起こり肝炎も鎮静化しやすく，また肝発癌も少ないことがわかっている．このため経過観察もHBV genotypeを意識して行うべきである．

インターフェロン療法の実際●

1986年にIFN-β，1988年にIFN-αがHBe抗原陽性，DNAポリメラーゼ陽性のB型慢性肝炎に対して4週間の短期の投与期間で保険適応となったため，海外ではわが国とは遺伝子型，感染様式は異なるが約25～40%のHBe抗原・抗体のSC率に対して，わが国ではSC率は約10%に過ぎなかった．その後2002年から24週間の投与が保険適応となり，わが国でも24週間の長期投与が可能となりSC率も向上した．B型慢性肝炎に対するIFNの治療効果は人種差，水平感染と垂直感染の差，HBV genotypeによる差があり，海外のデータをそのままわが国のB型慢性肝炎症例に当てはめるのは困難である．わが国でのB型慢性肝炎に対するIFN長期投与のまとまった成績は少なく，治療効果の判定基準も定まっていないが，若年者，IFN開始時のHBV-DNA量低値，ALT高値例，genotype AまたはBに有効例が多いと報告されている．ガイドラインでもgenotype A，Bは，35歳以上でもIFNの効果が高率であることから，可能なかぎりIFNを第一選択にすることが望ましいとされている．

IFN長期投与の肝発癌を含めた長期的な有用性についての報告は少なく，今後の明らかにされて行くべき課題である．

HBe抗原陰性例に対するIFN治療効果は，IFN投与中の抗ウイルス効果，肝炎鎮静化は良

0910-1551/11/ ¥100/頁 /JCOPY

- B型肝炎の治療目標はHBVの持続的な増殖抑制である.
- 35歳未満のB型肝炎ではIFN治療が第一選択であり，長期投与が望ましい.
- 核酸アナログ製剤は副作用が軽微で，抗HBV効果は強力であるが，長期投与が必要である.

好であるが，問題は治療後の肝炎再燃例が非常に多いことである．保険適応の枠を越えた長期投与が必要となる場合もある．

わが国ではまだ未承認であるが欧米からPEG-IFNα-2aの単独あるいはラミブジンとの併用の成績が報告されており，ラミブジン単独に優る効果が示されている[1]．わが国でもIFN-αを対照とした臨床試験が行われており，国内での成績が今後明らかにされる．

IFNは治療中にSCが得られれば，中止後も治療効果が持続する症例が多いが，HBe抗原陽性でHBV-DNA量の多い(＞7 log copies/ml)症例ではe抗原の陰性化やHBV-DNAの十分な低下が得られる確率が低く，これらの症例に対しては若年者であっても核酸アナログ＋sequential療法[2]を検討する必要があると思われる．

核酸アナログ治療の実際

2000年11月のゼフィックス®(ラミブジン)の承認以来，核酸アナログ製剤がB型慢性肝炎治療に広く用いられるようになっている．核酸アナログはDNA polymeraseの天然の基質と競合し酵素活性を阻害するという共通の作用を持っている．わが国ではラミブジンに加えアデホビル，エンテカビルが使用可能となっている．核酸アナログ製剤はIFNと異なり内服製剤であり，副作用が非常に軽微であり，抗ウイルス効果が非常に強力であるという特徴を有している．一方，核酸アナログ製剤の共通の問題点として① 耐性ウイルスの出現，② 投与中止後の肝炎の再燃，③ 胎児への安全性が確立されていない点があげられる．

1. 核酸アナログ治療の経過

核酸アナログ治療は一般的に長期間の治療となる．内服治療開始後，通常は速やかにHBV増殖が抑制され血中のHBV-DNAは減少する．抗ウイルス効果が順調に発揮されれば，やがてHBe抗原・抗体のSCが起こる．この段階で症例によっては核酸アナログ投与の中止が考慮される．さらに投与を継続することにより一部の症例ではHBs抗原の陰性化，さらにはHBs抗体が出現しウイルス学的治癒に至る(**図1**)．しかしながらHBs抗原陰性化まで至る症例は非常に少数であり，当科の3年以上核酸アナログ投与を投与した190例中5例にすぎない．

現在，国内では3種類の核酸アナログ製剤が使用可能であるが，耐性株の出現頻度がラミブジンでは1年17%，2年42%，3年53%，アデホビルでは1年0%，2年3%，3年11%であるのに対して，エンテカビルでは1年0%，2年0%，3年3.3%であり，ガイドラインではエンテカビルを第一選択の薬剤としている．エンテカビルのHBV増殖抑制作用は強力で当科のデータでは1年で約90%，2年，3年で約95%の症例で血中HBV-DNAが陰性化または2.1 log copies/ml未満(TaqMan HBV)に低下し，大部分の症例でALTの正常化も得られている(**図2**)．しかし少数ではあるがHBV-DNAの減衰は認めるが2.1 log copies/ml未満まで低下しない症例も存在し，これらの症例では将来的に耐性株が出現する可能性も考えられる．

2. 核酸アナログの投与中止

当初ラミブジンが主に使用されていた時期から核酸アナログ長期投与による抵抗株の出現問題，さらに若年症例のdrug freeの希望などにより，一定の治療効果のもと(HBV-DNA陰性かつHBe抗原陰性)に核酸アナログ製剤の中止が試みられている．しかしながら当科の成績でもラミブジン投与中止1年後には約70%の症例でHBV再増殖に伴い肝炎が再燃しており，核酸アナログの投与中止時期の設定は困難であった．HBV-DNA