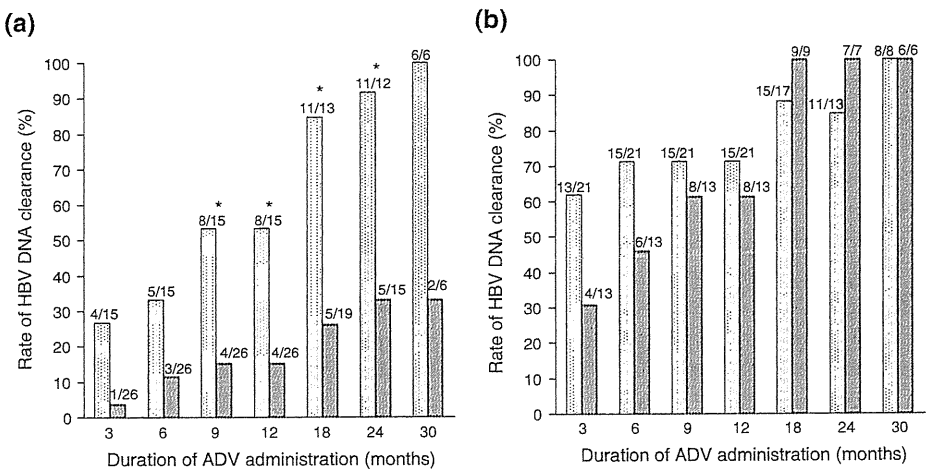

Fig. 2 Rates of HBV DNA clearance during ADV therapy in addition to LAM according to HBV DNA at baseline in **a** HBeAg-positive CHB patients and **b** HBeAg-negative CHB patients. $*P < 0.05$ between patients with low (≤7.0 logcopies/ml) and high (>7.0 logcopies/ml) HBV DNA. *Dotted bars* Patients with HBV DNA ≤7.0 logcopies/ml at baseline, *hatched bars* patients with HBV DNA >7.0 logcopies/ml at baseline

HBeAg-negative CHB patients in relation to baseline HBV DNA. In the case of HBeAg-positive CHB patients (Fig. 2a), the rates of HBV DNA clearance were 33% (5/15) at 6 months, 53% (8/15) at 12 months, and 92% (11/12) at 24 months in patients with low viremia (baseline HBV DNA ≤7.0 logcopies/ml). By contrast, the frequencies of HBV DNA clearance were only in 12% (3/26) at 6 months, 15% (4/26) at 12 months, and 33% (5/15) at 24 months in patients with high viremia (baseline HBV DNA >7.0 logcopies/ml). A significant difference ($P < 0.05$) in the frequency of HBV DNA clearance was observed between patients with low and high viremia at 9, 12, 18, and 24 months of treatment. In the case of HBeAg-negative patients (Fig. 2b), the rates of HBV DNA clearance were 71% (15/21) at 6 months, 71% (15/21) at 12 months, and 85% (11/13) at 24 months in patients with low viremia (baseline HBV DNA ≤7.0 logcopies/ml). The frequencies of HBV DNA clearance were 46% (6/13) at 6 months, 62% (8/13) at 12 months, and 100% (7/7) at 24 months in patients with high viremia (baseline HBV DNA >7.0 logcopies/ml). No significant differences were observed in the frequency of HBV DNA clearance between patients with low and high viremia. According to these findings, the relevance of lower baseline HBV DNA for achieving a better antiviral effect was evident only in HBeAg-positive patients, but not in HBeAg-negative ones in ADV therapy added to LAM treatment for LAM-resistant CHB.

Discussion

This study investigated factors affecting the antiviral efficacy of ADV therapy added to ongoing LAM treatment in LAM-resistant CHB patients. Therapeutic efficacy was assessed as the presence or absence of VR. Both univariate and multivariate analyses revealed that lower baseline HBV DNA and negative HBeAg were strong factors associated with a better therapeutic response. Another significant factor revealed by multivariate analysis was high ALT, although it was weaker than the other two factors. In previous investigations, female gender, lower baseline HBV DNA, negative HBeAg, higher ALT, and genotype D rather than A have been reported to contribute to better VRs to ADV therapy in nucleos(t)ide-naïve and LAM-resistant CHB patients [17–21]. Our results agreed partially with them. The present study, as well as previous studies [18, 19], also revealed that a high baseline ALT may be a determining factor for a better response to ADV therapy in addition to LAM treatment in LAM-resistant CHB. This may be because the host immune response against viral antigens induced by active breakthrough hepatitis has a favorable antiviral effect during ADV therapy. In this study, however, a low baseline viremic level was shown to be a stronger factor than high baseline ALT. The baseline ALT level was the third factor contributing to VR. Therefore, in LAM-resistant CHB, ADV administration should be started before the flare-up of ALT elevation, especially in patients with severe liver disease such as cirrhosis.

In LAM-resistant patients, the HBV DNA level is low during the initial phase, but increases with time, leading to the onset of breakthrough hepatitis. Thus, in ADV therapy added to LAM treatment for LAM-resistant-CHB, the baseline HBV DNA level varies with the observation period after the emergence of LAM resistance. A previous report on Italian HBeAg-negative CHB patients showing LAM resistance revealed that patients with low viremia and normal ALT tended to respond to ADV therapy in addition to LAM treatment better than those with high viremia and abnormal ALT [17]. In the present study conducted in Japan, a genotype C-endemic area, such a close relationship between lower baseline HBV DNA and better therapeutic response was remarkable in

HBeAg-positive patients but not in HBeAg-negative ones. Our finding suggests that, in LAM-resistant CHB, ADV should be added before the HBV DNA begins to increase markedly, especially in HBeAg-positive patients.

In this study, none of the 75 patients showed virological breakthrough after the beginning of ADV administration. All displayed more than 1 log reduction of HBV DNA at 12 months of ADV treatment. This indicates that our patients may not have produced viruses resistant to both LAM and ADV. The emergence of resistant viruses has been reported to be rare in combination therapy using LAM and ADV for LAM-resistant CHB patients, although recent studies have found the existence of a virus resistant to both drugs [22, 23]. The rtA181V/T/S mutation has been reported to confer cross resistance to LAM and ADV [22, 23]. In ADV monotherapy for nucleos(t)ide analog-naïve CHB patients, the absence of HBV DNA reduction to <4 logcopies/ml at 24 weeks of treatment has been reported to be related to the higher emergence of a ADV-resistant virus [24], as is the case in LAM monotherapy [25]. In ADV therapy added to LAM treatment in LAM-resistant CHB patients, the poor response during the initial phase may lead to the development of virus resistance to LAM and ADV as well. From this point of view, the addition of ADV to ongoing LAM treatment before the elevation of HBV DNA may be beneficial in LAM-resistant CHB patients to avoid the development of a multi-drug-resistant virus. Recently, some investigators have reported that tenofovir disoproxil fumarate is effective against a virus resistant to both LAM and ADV [22, 23], but it has not yet been approved for clinical use.

Our results conclusively showed that, with ADV therapy added to LAM treatment for LAM-resistant CHB patients, lower baseline HBV DNA and negative HBeAg contributed to a better antiviral effect. After the emergence of LAM resistance, ADV should be added before the marked elevation of HBV DNA in order to attain better antiviral efficacy, especially in HBeAg-positive patients.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Lamivudine-to-entecavir switching treatment in type B chronic hepatitis patients without evidence of lamivudine resistance

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Abstract
Purpose A considerable number of chronic hepatitis B (CH-B) patients remain under continuous lamivudine treatment, although switching treatment to entecavir could be beneficial. We investigated the antiviral efficacy of switching treatment to entecavir in CH-B patients without apparent evidence of lamivudine resistance during the preceding lamivudine treatment.
Methods Forty-four CH-B patients, who underwent lamivudine treatment for more than 6 months and showed no evidence of lamivudine resistance, switched to entecavir. Serial changes in hepatitis B virus (HBV) DNA were correlated with the patients' baseline HBV DNA at the commencement of entecavir administration. The entecavir-resistant substitution was examined by PCR-direct sequencing. The median follow-up period of entecavir treatment was 20 (10–23) months.
Results All 31 patients with baseline HBV DNA <2.6 logcopies/ml maintained HBV DNA-negative status during entecavir treatment. Of seven patients having HBV DNA of 2.6–<4.0 logcopies/ml, all achieved undetectable HBV DNA at the end of follow-up. As for six patients having HBV DNA ≥4.0 logcopies/ml, three patients achieved undetectable HBV DNA, whereas virological breakthrough was observed in one patient at month 15. An entecavir-resistant virus having rtM204V, rtL180M and rtS202G substitutions was detected in this patient.
Conclusions The lamivudine-to-entecavir switching treatment may be generally recommendable in CH-B patients without evidence of lamivudine resistance during

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the preceding lamivudine treatment. However, great care should be taken with respect to the emergence of entecavir-resistance, especially in patients who do not respond well to the preceding lamivudine treatment.

Keywords Chronic hepatitis B · Lamivudine resistance · Entecavir-resistance

Introduction

Nucleos(t)ide analogs have been accepted as useful agents for suppressing hepatitis B virus (HBV) replication and disease progression in patients with type B chronic hepatitis (CH-B). Lamivudine, the first approved nucleoside analog, has been shown to provide short-term benefit for CH-B patients with respect to the reduction of HBV DNA, normalization of alanine aminotransferase (ALT) and improvement of liver histology [1, 2]. However, a serious shortcoming of lamivudine is the high incidence of drug resistance during long-term treatment. The detection rate of lamivudine resistance has been reported to be 24% at 1 year and 70% at 4 years of treatment [3]. Lamivudine resistance is caused by an rtM204V/I substitution within the reverse transcriptase domain of HBV polymerase gene [4–6]. An rtL180M substitution frequently emerges as a "replication-compensatory" one with the "resistance-causative" rtM204V/I substitution [4–7]. The emergence of lamivudine-resistant mutant HBV leads to the elevation of HBV DNA ("virological breakthrough") and the subsequent increase of ALT ("breakthrough hepatitis"), resulting in disease progression. Adefovir dipivoxil and tenofovir disoproxil fumarate have been shown to be effective in both nucleos(t)ide analog-naïve and lamivudine-resistant CH-B patients [8–13].

Recently, entecavir has been demonstrated to exert antiviral efficacy in both nucleos(t)ide analog-naïve and lamivudine-refractory CH-B patients [14–16]. The frequency of entecavir-resistance has been reported to be less than 1% at 4 years of treatment in nucleos(t)ide analog-naïve CH-B patients [17]. On the other hand, in switching treatment to entecavir for lamivudine-refractory CH-B patients, most of whom developed lamivudine resistance during the preceding lamivudine therapy, the cumulative probability of entecavir-resistance has been reported to be no less than 40% at 4 years of treatment [17]. Entecavir-resistance has been shown to be established by amino acid substitution(s) at rt184, rt202 and/or rt250 along with the lamivudine-resistant rtM204V and rtL180M substitutions [18]. In the case of nucleos(t)ide analog-naïve patients, the requirement of at least three amino acid substitutions serves as a high genetic barrier to entecavir-resistance. By contrast, in the case of lamivudine-resistant patients, a lower genetic barrier results in higher incidence of entecavir-resistance because two amino acid substitutions, rtM204V and rtL180M, already exist from the preceding lamivudine treatment. The reduced susceptibility to entecavir of the lamivudine-resistant virus compared with the wild-type virus is also a reason for the higher emergence rate of entecavir-resistance in lamivudine-resistant patients than in nucleos(t)ide analog-naïve ones [19].

Although lamivudine is not currently recommended as a first-line drug for nucleos(t)ide analog-naïve CH-B, a considerable number of CH-B patients are under continuous treatment with lamivudine. In these patients, the switch to entecavir treatment could be advantageous over continuation of lamivudine treatment by offering stronger antiviral efficacy and less chance of drug resistance. With respect to the manner of emergence of entecavir-resistance, switching a patient's treatment may be more appropriate before the appearance of lamivudine resistance than after its development. However, the usefulness of lamivudine-to-entecavir switching treatment has not been assessed in CH-B patients without apparent evidence of lamivudine resistance.

This led us to investigate the antiviral efficacy and emergence of entecavir-resistance in CH-B patients who showed no evidence of lamivudine resistance during the preceding lamivudine treatment and underwent the switching treatment to entecavir.

Patients and methods

Patients

This study included 44 consecutive CH-B patients from 10 institutions in the Osaka area of Japan (Otemae Hospital, Sumitomo Hospital, Osaka Police Hospital, Suita Municipal Hospital, Yao Municipal Hospital, Osaka Rousai Hospital, Ikeda Municipal Hospital, National Hospital Organization Osaka National Hospital, Itami City Hospital and Osaka University Hospital) who underwent continuous lamivudine treatment (100 mg/day) for more than 6 months and showed no apparent evidence of lamivudine resistance. Before starting the preceding lamivudine treatment, all patients had abnormal ALT, positive hepatitis B surface antigen (HBsAg) and a detectable level of HBV DNA according to PCR-based assay (Amplicor HB Monitor, Roche Diagnostics) or branched DNA assay (Quantiplex HBV DNA, Chiron). None of them showed evidence of dual infection with hepatitis C virus or human immunodeficiency virus, or other forms of liver diseases such as alcoholic liver disorder, autoimmune hepatitis and drug-induced liver injury. The total duration of the preceding lamivudine treatment ranged from 6 to 73 (median, 14)

months. The absence of lamivudine resistance was defined by no detection of the rtM204V/I substitution as measured by the PCR–enzyme linked minisequence assay (ELMA) (Sumitomo Metal Industries) [20] for 33 patients, or by the lack of virological breakthrough as judged by more than 1 log increment in HBV DNA from the nadir for the remaining 11 patients. All of the 44 patients switched to 0.5 mg/day of entecavir administration. After the beginning of entecavir treatment, liver function tests and HBV markers were measured at 1- to 2-month intervals. When virological breakthrough was observed during follow-up, entecavir-resistance-associated mutations were examined by means of a PCR-direct sequencing method. The follow-up period of entecavir treatment ranged from 10 to 23 (median 20) months.

Baseline characteristics of the patients

At the commencement of switching treatment to entecavir, the 28 males and 16 females were aged 33–79 (median 59) years. Seventeen patients (39%) tested positive for hepatitis B e antigen (HBeAg), and antibody against HBeAg (anti-HBe) developed in all of the 27 HBeAg-negative patients. Among the 27 HBeAg-negative patients, four achieved HBeAg clearance during the preceding lamivudine treatment. HBV DNA at baseline varied among patients from <2.6 to 5.2 logcopies/ml. The baseline ALT ranged from 11 to 78 (median 25) IU/l. Regarding the liver diseases of the patients, 27 (61%) showed features of chronic hepatitis, 11 (25%) of liver cirrhosis and six (14%) of hepatocellular carcinoma (HCC) according to liver biopsy and/or abdominal imaging procedures. HBV genotype was examined for 14 patients, and all of them had HBV genotype C, the most predominant genotype in Japan. Informed consent was obtained from all patients.

Serological and virological markers of HBV

HBsAg, HBeAg and anti-HBe were determined by chemiluminescent immunoassay. HBV DNA was measured by the PCR-based method (Amplicor HBV monitor, Roche Diagnostics) whose lower detection limit is 2.6 logcopies/ml. Lamivudine-resistant rtM204V/I substitution was examined by the PCR–ELMA method (Sumitomo Metal Industries) (20), which is capable of detecting the mutant virus in a mixed viral population if it is present at more than 10% of the total population. The entecavir-resistance-associated substitutions and HBV genotype were determined by a PCR-direct sequencing method. As for oligonucleotide primers for PCR reaction, the outer primer sets were BF5 (5′-AAG AGA CAG TCA TCC TCA GG-3′, nt 3183–3202) and BR1s (5′-AAA AAG TTG CAT GGT GCT GG-3′, nt 1825–1806), and the inner primer sets were BF6 (5′-CCT CCA ATT TGT CCT GGC TA-3′, nt 350–369) and BR8 (5′-TTG CGT CAG CAA ACA CTT GG-3′, nt 1195–1176). After DNA extraction, the DNA sample was subjected to the PCR reaction for 35 cycles (denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min) using the inner primer set, followed by a final extension at 72°C for 10 min. If amplification was not successful by the single PCR reaction, the nested PCR was conducted; the first round PCR was done using the outer primer sets for 35 cycles, and the aliquot of the product was used for the second round PCR for 30 cycles using inner primer sets. All sequencing reactions of the PCR products were carried out using the BigDye Terminater Ver. 3.1 Cycle Sequencing Kit, and 3100 or 3730 Genetic Analyzer (Applied Biosystems), which allowed determination of the amino acid sequences of rt85-344. For determining the HBV genotype, nucleotide sequences obtained in each of the patients were aligned along with representative HBV strains of genotype A–H, and a phylogenetic tree was constructed in the homepage of DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp).

Statistical analysis

Statistical analysis for group comparison was performed by Fisher's exact probability test and Mann–Whitney's non-parametric U test using the SPSS version 15.0J software (SPSS Inc, Chicago, IL). A p value of less than <.05 was considered to be significant.

Results

Classification of patients who underwent lamivudine-to-entecavir switching treatment according to baseline HBV DNA

The 44 CH-B patients who underwent the switching treatment from lamivudine to entecavir were first classified according to their baseline HBV DNA at the commencement of entecavir administration. HBV DNA was not detectable (<2.6 logcopies/ml) in 31 patients (70%) at baseline. Seven patients (16%) had baseline HBV DNA of 2.6–<4.0 logcopies/ml. In the remaining six patients (14%), the baseline HBV DNA was ≥4.0 logcopies/ml. When patient clinical characteristics were compared among the three patient groups (Table 1), nine (29%) of the 31 patients with baseline HBV DNA <2.6 copies/ml tested positive for HBeAg at the commencement of switching treatment to entecavir, compared with five of the six (83%) patients with baseline HBV DNA ≥4.0 copies/ml ($p < .05$). Gender ratio, age, ALT at baseline, liver disease, duration of the preceding lamivudine treatment and

Table 1 Patient clinical characteristics and the therapeutic efficacy in 44 CH-B patients in relation to their baseline HBV DNA

	Baseline HBV DNA		
	<2.6 logcopies/ml ($n = 31$)	2.6–<4.0 logcopies/ml ($n = 7$)	≥4.0 logcopies/ml ($n = 6$)
At the commencement of switching treatment to entecavir			
Gender (male/female)	19/12	5/2	4/2
Age (years)	60 (35–79)[a]	65 (41–69)	55 (33–65)
HBeAg (positive/negative)	9/22	3/4	5/1[b]
HBV DNA (logcopies/ml)	<2.6	3.1 (2.6–3.6)[c]	4.6 (4.0–5.2)[c,d]
rtM204V/I mutation (absence/NT)	23/8	5/2	5/1
ALT (IU/l)	25 (11–64)	31 (13–46)	20 (17–78)
Chronic hepatitis/cirrhosis/HCC	19/7/5	4/2/1	4/2/0
Follow-up period of entecavir treatment (months)	19 (10–23)	19 (10–22)	20 (16–22)
The rate of undetectable HBV DNA level during follow-up	31 (100%)	7 (100%)	3 (50%)[c]
Emergence of entecavir-resistance during follow-up	0 (0%)	0 (0%)	1 (17%)
At the commencement of preceding lamivudine treatment			
HBeAg (positive/negative)	12/19	4/3	5/1
HBV DNA (logcopies/ml)	6.5 (4.3–7.6‹)	6.6 (6.2–7.6‹)	7.6‹ (5.9–7.6‹)
Duration of preceding lamivudine treatment (months)	15 (6–73)	10 (7–42)	9 (8–32)

NT not tested

[a] Values are expressed as median (range)

[b] $p < .05$ versus baseline HBV DNA <2.6 logcopies/ml group

[c] $p < .01$ versus baseline HBV DNA <2.6 logcopies/ml group

[d] $p < .01$ versus baseline HBV DNA of 2.6–<4.0 logcopies/ml group

follow-up period of entecavir treatment did not differ among the three groups. Also, there was no significant difference in HBV DNA and the frequency of positive HBeAg at the commencement of preceding lamivudine treatment among them.

Antiviral efficacy and drug resistance in lamivudine-to-entecavir switching treatment in relation to baseline HBV DNA

Next, we investigated serial changes in HBV DNA after the switch from lamivudine to entecavir treatment in CH-B patients in relation to the baseline HBV DNA. All 31 patients with baseline HBV DNA <2.6 logcopies/ml maintained undetectable HBV DNA during the follow-up period of entecavir treatment. Figure 1 shows the longitudinal evaluation of HBV DNA during the switching treatment to entecavir in patients with a detectable level of baseline HBV DNA. In patients having baseline HBV DNA of 2.6–<4.0 logcopies/ml (Fig. 1a), all of the seven patients achieved sustained undetectable HBV DNA during follow-up, although HBV DNA was transiently detected in one patient. As for patients having baseline HBV DNA ≥4.0 logcopies/ml (Fig. 1b), three (50%) of the six patients achieved sustained undetectable HBV DNA during follow-up. In two patients, HBV DNA was not cleared entirely, but declined to 2.9 and 2.7 logcopies/ml at month 18, respectively. In sequencing analysis at that time, the former patient had the lamivudine-resistant rtM204I substitution, although it was not detected by the PCR–ELMA assay at the start of entecavir treatment. The latter patient had no drug resistance-associated substitutions. In the sixth patient, HBV DNA decreased initially, but virological breakthrough was seen at month 15. The entecavir-resistant virus was detected after virological breakthrough. The detailed disease course of the entecavir-resistant patient is described below. As for the relationship of baseline HBV DNA to the frequency of undetectable HBV DNA, HBV DNA was cleared more frequently in patients with baseline HBV DNA <2.6 logcopies/ml than in those with baseline HBV DNA ≥4.0 logcopies/ml (100 vs. 50%, $p < .01$) (Table 1).

Serial changes in ALT during lamivudine-to-entecavir switching treatment were further examined. Among the 31 patients with baseline HBV DNA <2.6 logcopies/ml, the baseline ALT was within the normal range (≤40 IU/l) in 27 patients, 24 of whom showed sustained ALT normalization during follow-up. In the remaining three patients, ALT became slightly abnormal (≤60 IU/l) during follow-up. As for four patients with abnormal baseline ALT, the level was normalized in three, whereas a slight elevation of ALT (≤60 IU/l) continued in one during follow-up.

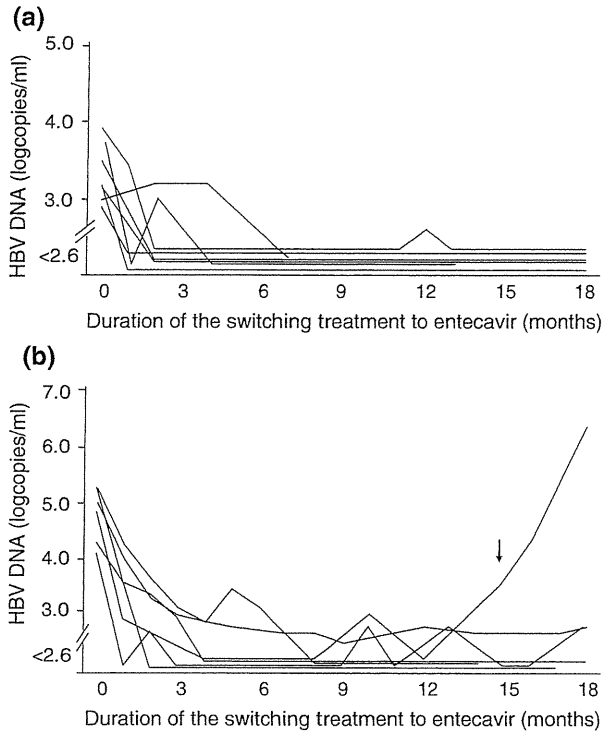

Fig. 1 Changes in HBV DNA after commencement of switching treatment from lamivudine to entecavir in CH-B patients with baseline HBV of (**a**) 2.6–<4.0 logcopies/ml and (**b**) ≥4.0 logcopies/ml. The *black arrow* indicates the time point of virological breakthrough

Among the 13 patients having a detectable level of baseline HBV DNA, five patients (three with baseline HBV DNA of 2.6–<4.0 logcopies/ml and two with baseline HBV DNA ≥4.0 logcopies/ml) had abnormal ALT at baseline but showed ALT normalization during follow-up. In the remaining eight patients, ALT continued to be normal from the beginning of entecavir treatment.

Disease course of the CH-B patients showing entecavir-resistance during lamivudine-to-entecavir switching treatment

The disease course of the entecavir-resistant patient is shown in Fig. 2. This patient was a 33-year-old HBeAg-positive male, whose liver biopsy showed features of chronic hepatitis. He underwent the preceding lamivudine treatment for 8 months. HBV DNA decreased from >7.6 to 4.6 logcopies/ml, and ALT was normalized during the lamivudine therapy. The rtM204V/I substitution was not detected before the switch to entecavir treatment by the PCR–ELMA analysis. After the commencement of entecavir treatment, HBV DNA was cleared at month 5. However, virological breakthrough was seen at month 15, and HBV DNA was further increased to 6.1 logcopies/ml at month 18. The sequencing analysis at month 18 revealed the rtM204V, rtL180M and rtS202G substitutions. Two additional substitutions, rtL267M and rtQ316H, were also found, when the amino acid sequences were compared with three representative genotype C HBV isolates (Genbank accession nos. V00867, X01587 and D00630) [21–23]. Breakthrough hepatitis was not evident after the emergence of entecavir-resistant mutant virus. The sequencing analysis also revealed that he was infected with HBV of genotype C.

Discussion

Entecavir treatment has been shown to exhibit more powerful antiviral efficacy and less frequent drug resistance than lamivudine treatment in nucleos(t)ide analog-naïve CH-B patients [14, 15, 17]. Entecavir is also effective in patients showing lamivudine resistance during the preceding lamivudine treatment, but its efficacy is limited due to the higher incidence of entecavir-resistance, compared with nucleos(t)ide analog-naïve ones [16, 17]. This is because entecavir-resistance is established based on two lamivudine-resistant substitutions, rtM204V and rtL180M, and additional mutation(s) occurring at rt184, rt202 and/or rt250 [18]. A considerable number of CH-B patients remain under continuous lamivudine treatment, while the lamivudine-to-entecavir switching treatment could yield a practical benefit. The switching treatment may be more promising for patients before the appearance of lamivudine resistance than after its development. In the present study, we investigated the efficacy of lamivudine-to-entecavir switching treatment in CH-B patients without apparent evidence of lamivudine resistance during the preceding lamivudine treatment.

We evaluated the antiviral efficacy of the switching treatment to entecavir in relation to the baseline HBV DNA at the commencement of the entecavir administration. In all patients having baseline HBV DNA <2.6 logcopies/ml, who revealed a good response to the preceding lamivudine treatment, HBV DNA continued to be undetectable during the switching treatment to entecavir. Also, all patients having baseline HBV DNA of 2.6–<4.0 logcopies/ml achieved sustained undetectable HBV DNA during the follow-up period of entecavir treatment. Among six patients having baseline HBV DNA ≥4.0 logcopies/ml, who did not respond well to the preceding lamivudine treatment, HBV DNA was cleared in three during follow-up. Its reduction by up to 3.0 logcopies/ml was seen in two additional cases without emergence of the entecavir-resistant virus. Thus, the antiviral efficacy of the lamivudine-to-entecavir switching treatment was exhibited in almost all CH-B patients in parallel with that of the preceding

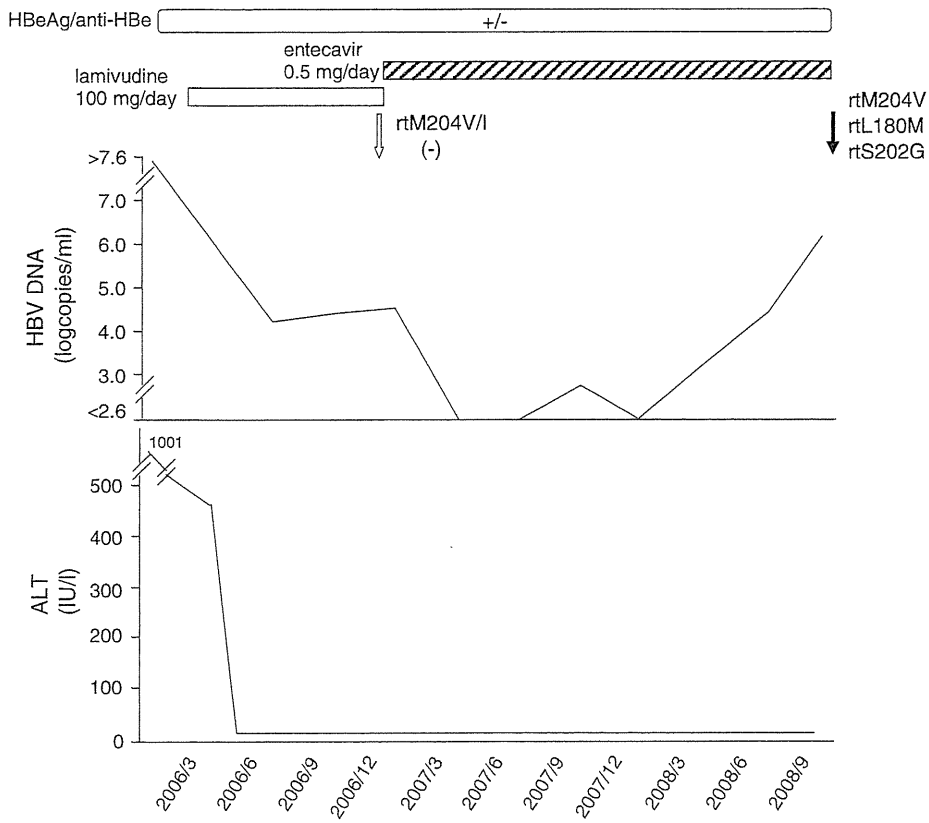

Fig. 2 Disease course of the CH-B patient showing entecavir-resistance during switching treatment to entecavir. The *white arrow* indicates the time point of the PCR–ELMA assay to detect rtM204V/I mutation, whereas the *black arrow* indicates the time point of the PCR-direct sequencing analysis

lamivudine treatment. In addition, the switching treatment to entecavir tended to yield a greater decrease in HBV DNA than the preceding lamivudine treatment. These results indicate that the switch from lamivudine to entecavir may be generally recommendable compared with continuation of lamivudine administration in CH-B patients without evidence of lamivudine resistance.

In this study, one of the six patients having baseline HBV DNA $\geq$4.0 logcopies/ml showed entecavir-resistance during the switching treatment to entecavir. It was probably due to the existence of an extremely small amount of lamivudine-resistant virus mixed with a predominant wild-type virus, which could not be detected by the sensitive PCR–ELMA assay at the start of the switch to entecavir treatment. It is speculated that, during entecavir treatment, the lamivudine-resistant virus having rtM204V and rtL180M substitutions may become predominant with time, followed by the establishment of entecavir-resistant virus via the additional rtS202G substitution. Compared to the low incidence of drug resistance in entecavir treatment for nucleos(t)ide analog-naïve CH-B patients [17], the entecavir-resistance may occur more frequently in the lamivudine-to-entecavir switching treatment for patients without evidence of lamivudine resistance. In particular, patients who do not achieve a good response to the preceding lamivudine treatment are speculated to have a higher risk for the development of entecavir-resistance in the switching treatment to entecavir, although it should be verified by further studies.

In conclusion, in CH-B patients receiving the continuous lamivudine treatment, it may be recommendable to switch to entecavir treatment before the appearance of lamivudine resistance. It may contribute to reducing the subsequent emergence of drug resistance. However, great care should be taken with respect to the emergence of entecavir-resistant virus after the switch to entecavir treatment, especially in patients who do not respond well to the preceding lamivudine treatment. Our retrospective study with a small number of patients and a short duration of follow-up cannot draw a definite conclusion but still provides some information about the clinical possibilities of the lamivudine-to-entecavir switching treatment. Further detailed investigation with a larger number of patients and a longer follow-up period may offer better understanding.

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B型肝炎

B型肝炎の抗ウイルス療法の実際

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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

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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

核酸アナログ治療ではHBV-DNAの抑制，ついでHBe抗原・抗体のセロコンバージョン，さらに長期の投与でHBs抗原陰性化が治療目標となる．第一選択の核酸アナログであるエンテカビルでは2～3年の投与で，約95%の症例でHBV-DNAの陰性化が得られる．

図1　核酸アナログ治療の長期経過

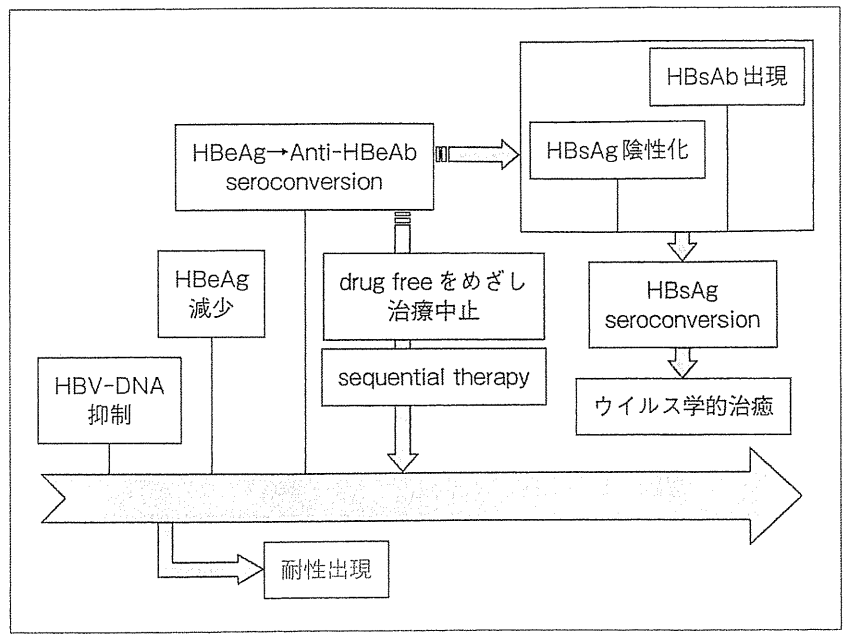
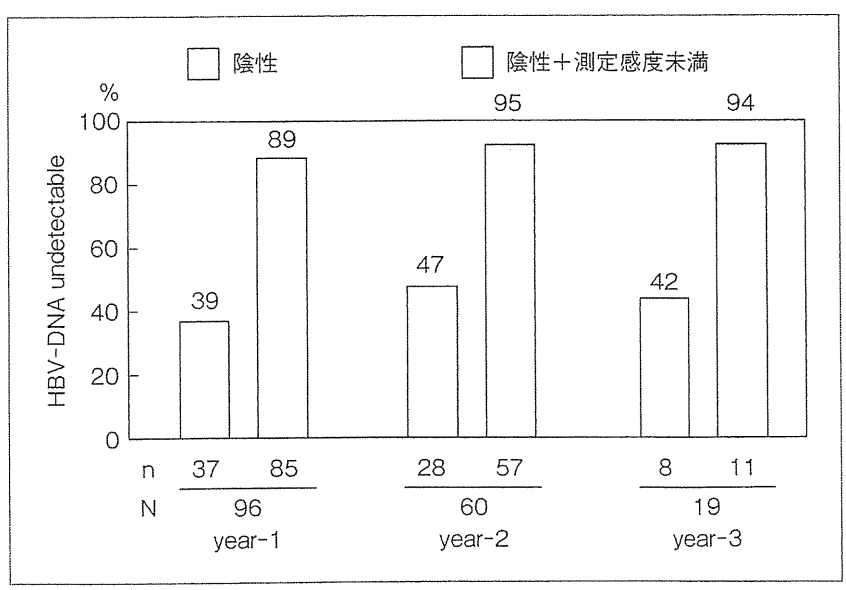


図2　HBV-DNA陰性化率(ETV)



とHBe抗原・抗体のみでは核酸アナログ投与中止時期決定のマーカーとしては十分ではなく，新たなマーカーとしてHBVコア関連抗原，HBs抗原が注目されている．核酸アナログ投与前はHBV-DNA，コア関連抗原，HBs抗原量は良好な相関を示すが，核酸アナログ投与後は減衰が乖離する(図3)．HBV-DNAは主に血液中のHBV量を反映し，コア関連抗原とHBs抗原は肝細胞中に存在するHBV量を反映していると考えられている．現在，厚生労働省の研究班ではこれらの

エンテカビル(naïve 例)では耐性ウイルスの出現はきわめて低率である．
核酸アナログ中止後の再燃を抑える目的で sequential therapy の効果が期待されている．

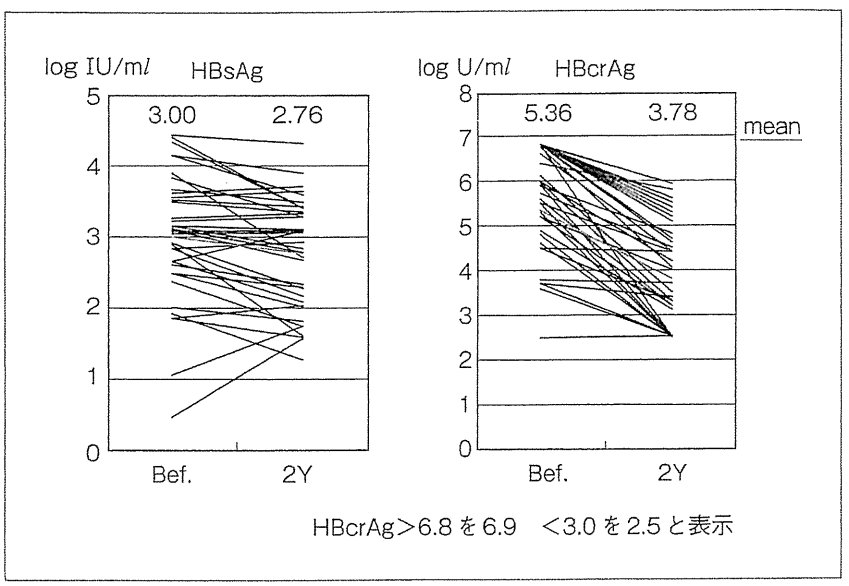


図3 ETV 投与2年後の HBsAg と HBcrAg の変化

マーカーを用いて核酸アナログ薬投与中止基準を策定中である．

3. 耐性ウイルス出現

エンテカビルが核酸アナログの第一選択薬となり，耐性株 HBV の出現による HBV 再増殖，さらに肝炎の再燃は激減した．しかしながら既存のラミブジン耐性ウイルス症例に対する対応が必要となっている．エンテカビルは rt180，204 のラミブジン変異に加えて rt184，202，250 のエンテカビルに特異的な変異が起こらなければ耐性ウイルスとはならず高い genetic barrier を有するが，ラミブジン耐性ウイルスではエンテカビルに特異的な変異が出現するだけでエンテカビル耐性(3年 36%)が出現する[3]．このためラミブジン耐性例に対してはラミブジン＋アデホビル併用療法が基本となる．ラミブジン＋アデホビル併用療法では HBV-DNA 陰性化は3年で 80% 程度にとどまり3年以降ではアデホビル耐性ウイルスの出現も認められる．当科ではこれらの症例に対してエンテカビル＋アデホビル併用療法を施行している(図4)．1年で平均約 1 log copies/ml の HBV-DNA 減衰を認めたが，効果不良な症例もありテノホビルなどの投与も検討する必要がある．今後は多剤耐性が疑われる症例に対しては耐性プロファイルを確認した上での薬剤選択が必要となる．

4. sequential 療法

Serfaty らが核酸アナログ療法に連続して IFN を投与後に治療を終了する sequential 療法の良好な効果を報告して以来，わが国でも同様の治療が行われているが核酸アナログ単独中止の成績を凌駕する成績は報告されていない．今年度のガイドラインでは，sequential 療法を行う場合は，核酸アナログ治療で HBe 抗原が陰性化(または陰性)症例で核酸アナログを十分投与し，HBV-DNA の陰性化期間が1年以上経過し，コア関連抗原も 4.0 logU/ml 以下の症例に行うのが望ましいと明記された．今後はガイドラインに沿った均一な集団でのわが国の sequential 療法の評価が望まれる．

5. pitfall

アデホビル投与時には定期的に腎機能(血清

アデホビル投与中は定期的に腎機能チェックが必要であり，
悪化例ではアデホビルの減量が必要である．

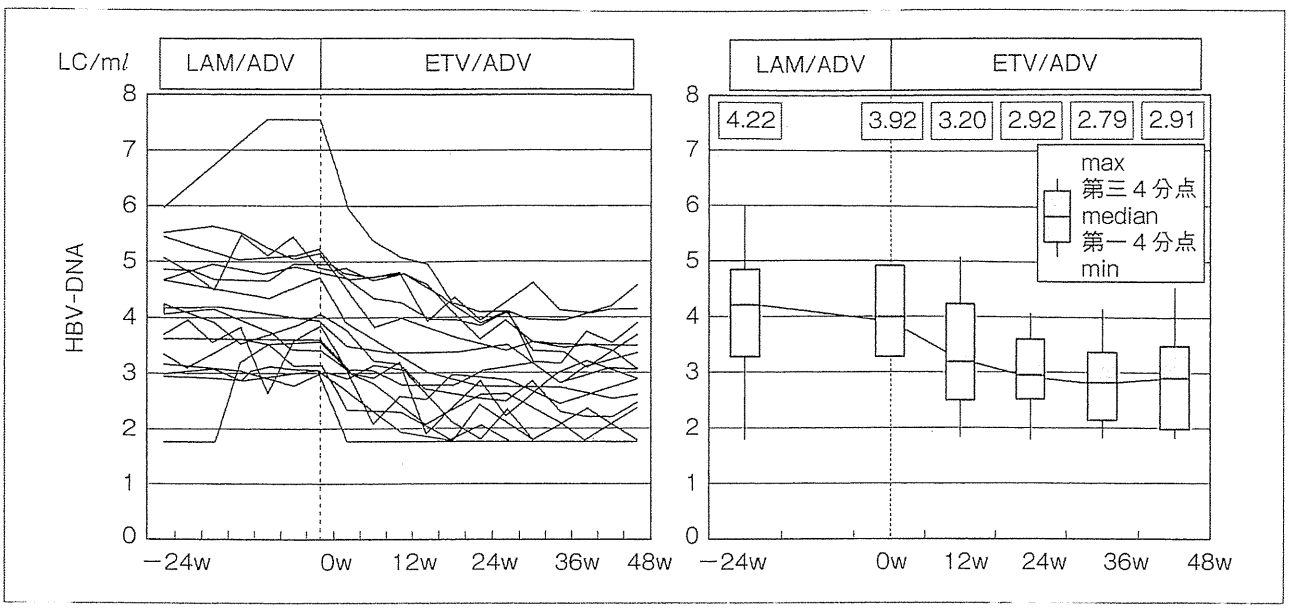


図４　LAM/ADV → ETV/ADV switch 例の HBV-DNA の推移

Cr，eGFR）の測定が必要である．腎機能低下例に対してはアデホビルを隔日投与，あるいは２日おきの投与とする．当科のアデホビル減量投与例では HBV-DNA の増加はほとんど認めていない．

HBV/HIV 重感染例は，エンテカビルの使用により HIV 耐性ウイルスが出現する可能性があるためエンテカビルは原則として使用すべきでない．したがってエンテカビル開始前にインフォームドコンセントを取得した上で HIV 抗体の測定を行うことが望ましい．

B 型肝硬変の抗ウイルス療法

肝硬変（代償性・非代償性）症例に対しては核酸アナログを投与する．ガイドラインでは適応 HBV-DNA 量 3 log copies/ml 以上と低い設定になっている．肝予備能の改善，肝発癌抑制が期待される．高度に肝予備能が低下した症例では回復が不良の症例もあり，可能な限り早期の導入が望ましい．

おわりに

B 型肝炎の自然経過には個人差が大きく，抗ウイルス療法導入後の HBV マーカーの変動も均一ではない．このため B 型肝炎の抗ウイルス療法に際しては個々の症例の治療適応と治療方法を十分吟味した上で治療にあたる必要がある．

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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*

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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.

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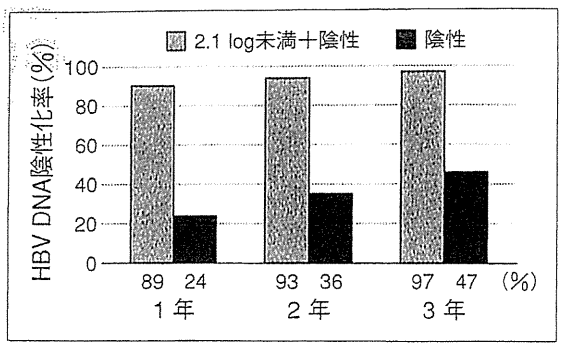


図1　HBV DNA陰性化率
TaqMan PCR法によるHBV DNA陰性化率の推移を示す．2.1 log未満と陰性例を加えた症例の割合は1年で89%，2年で93%，3年で97%，陰性例だけを抽出すると1年24%，2年36%，3年47%であった．

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

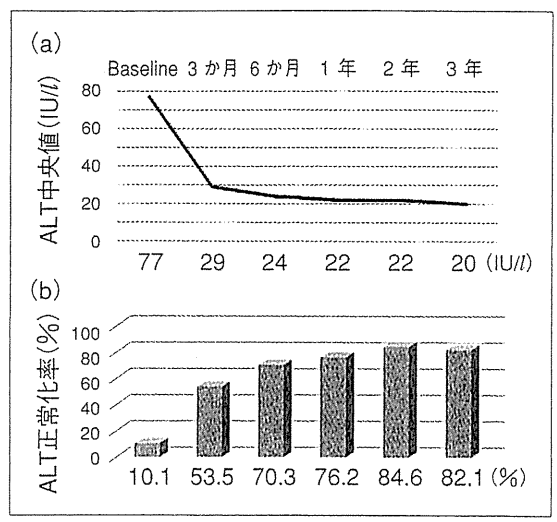


図2　ALT正常化率
(a)ALT中央値は治療開始時，3か月後，6か月後，1年後，2年後，3年後で，それぞれ，77 IU/*l*，29 IU/*l*，24 IU/*l*，22 IU/*l*，22 IU/*l*，20 IU/*l*であった．(b)ALT30 IU/*l*以下を正常として正常化率を算出した．治療開始時，3か月後，6か月後，1年後，2年後，3年後で，それぞれ，10.1%，53.5%，70.3%，76.2%，84.6%，82.1%であった．

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ー多変量解析ー

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

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)．

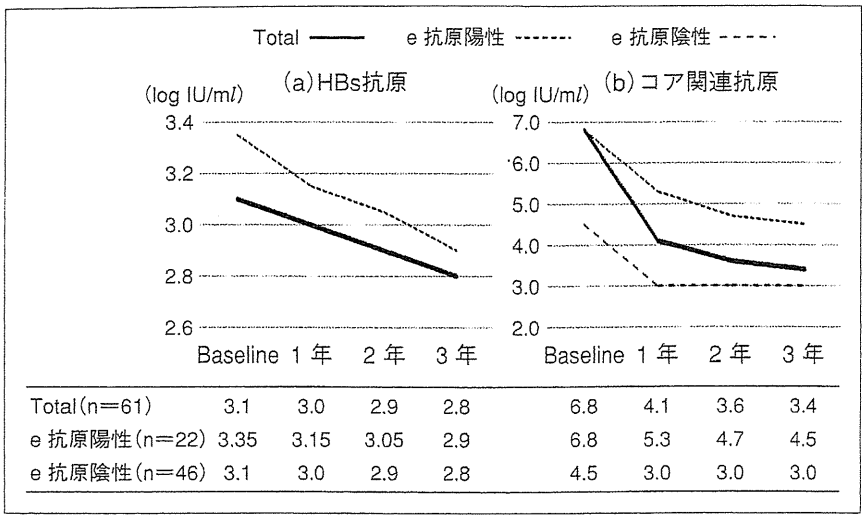
2．ALTの推移と陰性化率

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

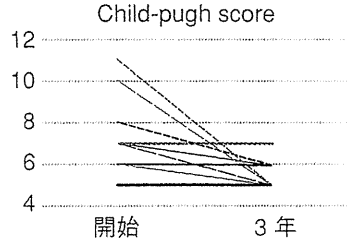


図4　肝予備能改善効果

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

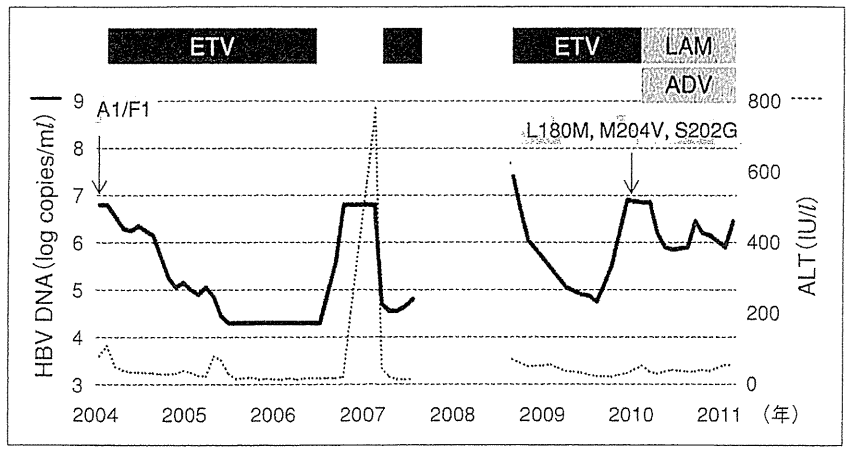


図5　ETV耐性が出現した1例
観察期間中央値31か月の経過でETV初回投与204例中1例(0.5%)でETV耐性(L180M, M204V, S202G)が出現した.

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6log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.

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