et al., 1999]. Another study reported a close correlation between the level of intrahepatic HCV-RNA and severity of steatosis [Rubbia-Brandt et al., 2000]. In the present study, no significant clinicopathological parameter (including serum HCV-RNA levels, fibrosis, and body mass index) was identified in non-sustained virological response patients with low-grade and high-grade steatosis. Thus, the results failed to establish a link between IFN-resistance and hepatocyte steatosis.

It is also not clear whether the virological characteristics of HCV play any pathogenic role in the derangement of lipid metabolism, and hence contribute to hepatocyte steatosis. Experiments conducted in vitro and in transgenic mice suggested that HCV core protein and NS5A region might be involved in the pathogenesis of lipid accumulation [Barba et al., 1997; Moriya et al., 1997; Shi et al., 2002]. On the other hand, Rubbia-Brandt et al. [2000] reported that steatosis might be a morphological expression of viral cytopathic effect in patients infected with HCV genotype 3, but that analysis of the HCV core protein failed to identify a sequence specially associated with the development of steatosis [Rubbia-Brandt et al., 2000]. No study has investigated the effects of HCV core protein and NS5A region on IFN efficacy in patients with hepatocyte steatosis. In this context, the relationship between amino acid substitutions in HCV core protein/NS5A region and the grade of hepatocyte steatosis was analyzed in the present study in IFN-resistant patients. However, the analysis showed no specific amino acid substitutions in these regions that could establish a role for hepatocyte steatosis in IFN-resistance. It should be noted, however, that the present study was based on a small group of patients and sequence analysis of the defined regions should be investigated in large-scale studies to confirm the present findings.

β IFN is rarely used and is not licensed outside Japan. It was reported previously that the type of IFN (α vs. β) is not a predictor of sustained virological response to IFN monotherapy in 394 patients infected with genotype 2a, based on multivariate analysis [Akuta et al., 2002], and accordingly when the present study was designed, it was thought that the type of IFN should not affect the outcome of patients infected with genotype 2a. Incidentally, based on data from Toranomon Hospital, the frequency of β IFN-related adverse events seems to be lower than those by α IFN, especially in elderly patients (unpublished data). Therefore, the use of β IFN rather than α IFN is recommended at least for elderly patients.

In conclusion, the present study of patients infected with HCV genotype 2a suggested that hepatocyte steatosis is possibly associated with excessive iron storage, and that it might be an important predictor of the efficacy of IFN monotherapy. Further studies should be performed to investigate whether hepatocyte steatosis associated with HCV genotype 2a might be also a predictor of other treatments, including IFN-ribavirin combination therapy and pegylated IFN. In this study, amino acid substitutions associated with IFN-resistance specific for hepatocyte steatosis could not be identified, and large-scale studies should be conducted to confirm the present findings. Further analysis of IFN-resistance mechanisms should be conducted in future studies taking into consideration pharmacokinetic, viral, and host-related factors.

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Virological and Biochemical Relapse after Discontinuation of Lamivudine Monotherapy for Chronic Hepatitis B in Japan: Comparison with Breakthrough Hepatitis during Long-Term Treatment

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Key Words
Chronic hepatitis B · Lamivudine monotherapy · Biochemical and virological relapse · Basic core promoter · YMDD motif mutant · Breakthrough hepatitis · Retreatment · HBV genotype

Abstract

Objective: Comparison of virological and biochemical relapse in patients with chronic hepatitis B, based on continuation or discontinuation of lamivudine monotherapy. ***Methods:*** In Japanese genotype C-dominant hepatitis B patients, 25 patients who stopped treatment at normal levels of alanine transferase (ALT) were retrospectively compared with 75 patients who continued treatment. Both groups were matched for age, sex, and observation period after start of treatment. We investigated the relapse rates, and evaluated predictive factors for relapse and efficacy of retreatment of the discontinuous group. ***Results:*** Virological and biochemical relapse occurred significantly earlier in the discontinuous than continuous group, and the peak levels and ratios of peak to pretreatment levels of serum bilirubin and ALT after relapse were not significantly different between the two groups. Multivariate analysis identified three independent factors at discontinuation of treatment associated with early biochemical relapse: HBeAg positivity, presence of liver cirrhosis, detection of basic core promoter mutant. Normalization of ALT levels with retreatment occurred in 62.5% of patients, but 2 HBeAg-positive patients retreated after the emergence of YMDD motif mutant developed severe relapse with hyperbilirubinemia. ***Conclusion:*** Our results in Japanese patients with genotype C-dominant hepatitis B suggest that discontinuation of lamivudine monotherapy, and retreatment after the emergence of YMDD mutant should be given attention.

Introduction

Lamivudine, an oral cytosine nucleoside analog clinically used for the treatment of chronic hepatitis B virus (HBV) infection, potently inhibits HBV replication by interfering with HBV reverse transcriptase activity [1–4], and results in a marked decrease of HBV DNA and serum transaminase levels, seroconversion of HBe antigen (HBeAg) to anti-HBe, and histopathological improvement [4–9].

The optimal duration of lamivudine administration for HBV-infected patients is still controversial for two main problems; drug resistance and sustainability of the response to treatment. In particular, there is a need to evaluate short-term treatment with respect to post-treatment safety and the sustainability of responses, and long-term treatment with regard to biochemical relapse (breakthrough hepatitis) associated with the emergence of YMDD motif mutant [10–17]. The American Association for the Study of Liver Diseases practice guidelines suggested that lamivudine could be discontinued in patients who had completed one year of treatment and had persistent HBeAg seroconversion on more than one occasion determined 2–3 months apart [18]. However, this is not completely evaluated in Japanese genotype C-dominant hepatitis B patients.

The present study was designed to deal with the following three issues: (1) To compare the virological and biochemical relapse rates according to the continuation or termination of lamivudine monotherapy, and to compare the risk of biochemical relapse after the termination of the treatment and breakthrough hepatitis during long-term treatment; (2) to determine the independent predictive factors at discontinuation of treatment that contributed to early biochemical relapse in discontinuous patients, and (3) to evaluate the efficacy of retreatment with lamivudine monotherapy.

Patients and Methods

Patients

Lamivudine therapy was provided to 394 consecutive patients with chronic hepatitis B who tested positive for HBs antigen at Toranomon Hospital between September 1995 and December 2002. Among these, 269 patients started lamivudine monotherapy at abnormal alanine transferase (ALT) levels (normal for ALT, 6–50 IU/l) and were able to achieve ALT normalization during treatment, and were enrolled in this retrospective study. The latter group consisted of 25 patients who stopped the lamivudine monotherapy during ALT normalization (discontinuous group) and 244 patients who did not stop the lamivudine monotherapy (continuous group), and the discontinuation or not of lamivudine during ALT normalization was selected at their own request. To compare the cumulative virological and biochemical relapse rates between the discontinuous group and continuous group, all 25 patients of the discontinuous group entered this study along with 75 patients of the continuous group. The latter group was selected from among the 244 because they matched patients of the discontinuous group with respect to sex, age, and observation period after the start of lamivudine monotherapy. They had been confirmed to have hepatitis by liver biopsies, were free of decompensated liver cirrhosis and hepatocellular carcinoma. Coinfection and superinfection with hepatitis A, C, and delta viruses, and human immunodeficiency virus were ruled out serologically or genomically using commercially available kits or conventional polymerase chain reaction (PCR)-based assays. None of the patients had a history of other liver diseases, such as autoimmune hepatitis, alcoholic liver disease, and metabolic disease.

Patients were given a dose of 100 mg of lamivudine once a day. The median period of treatment in the discontinuous group (0.72 years, range; 0.10–5.6 years) was significantly shorter than that of the continuous group (1.8 years, range; 0.71–7.6 years, $p < 0.0001$). The median observation period after the commencement of lamivudine therapy was not significantly different based on the matching of the two groups, and the periods were 2.1 years (range; 0.68–7.9 years) in the discontinuous group and 1.8 years in the continuous group (range; 0.71–7.6 years). In the discontinuous group, the median observation period after discontinuation of lamivudine therapy was 1.4 years (range; 0.15–6.7 years). With regard to the observation period, patients of the discontinuous group who received another course of lamivudine treatment for biochemical relapse and those of the continuous group who received additional interferon treatment for biochemical relapse, were treated as censored data at the time of lamivudine retreatment and additional interferon treatment in the statistical analysis of cumulative relapse rates. The clinical characteristics of enrolled patients are summarized in table 1, and those of discontinuation are shown in table 2.

Methods

Our study compared virological and biochemical relapse in continuous and discontinuous lamivudine monotherapy groups, and determined the independent predictive factors at discontinuation that contributed to early biochemical relapse in the discontinuous group. Furthermore, we also evaluated the efficacy of retreatment with lamivudine monotherapy. Patients in whom ALT levels became abnormal (>50 IU/l) after a period of ALT normalization were defined as biochemical relapsers. Patients in whom levels of HBV DNA re-elevated after the minimum levels, ignoring undetectable HBV DNA levels, were defined as virological relapsers. Especially, virological relapse during lamivudine treatment associated with the emergence of YMDD motif mutant were defined as DNA breakthrough, and biochemical relapse associated with DNA breakthrough were defined as breakthrough hepatitis. Clinical and laboratory assessments were performed at least once every month before, during, and after treatment. Adverse effects were monitored clinically by a detailed interview and medical examination at least once every month. Patient compliance with treatment was evaluated by a questionnaire.

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for various laboratory data including ALT levels, HBV DNA levels, and the presence of YMDD motif mutant. The serum samples were stored in aliquots at −80°C until use. HBs antigen and HBeAg/eAb were determined by radioimmunoassay (Abbott Diagnostics, Chicago, Ill., USA). HBV DNA was measured by transcription-mediated amplification and hybridization protect assay (TMA-HPA) (Chugai Diagnostica, Tokyo, Japan). The lower and upper limits of detection of TMA-HPA are 5×10^3 and 5×10^8 viral genomic equivalents (GE)/ml, respectively. HBV genotype was determined using a previously reported method [19, 20]. Antibody against HCV was detected with a third-generation enzyme-linked immunoassay (Ortho Diagnostic Japan, Tokyo). YMDD motif mutant was detected using the sensitive PCR-restriction fragment length polymorphism [21].

Liver biopsy specimens were obtained percutaneously or at laparoscopy using a modified Vim Silverman needle of 2 mm internal diameter (Tohoku University style, Kakinuma Factory, Tokyo). Each specimen was scored according to the system of Desmet et al. [22].

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and its subsequent amendments, and informed consent was obtained from each patient. The study was approved by the Human Ethics Committee of Toranomon Hospital.

Nucleotide Sequencing of HBV Basic Core Promoter (nt 1762/1764) and Precore (nt 1896)

Nucleotide sequences of HBV were compared with the prototype sequences of the HBV genotype C [19]. HBV DNA was extracted with a Smitest EX & R kit (Genome Science, Tokyo). Nucleic acids were amplified by nested PCR using the following primers. Nucleotide sequences of basic core promotor (BCP) nt 1762/1764 and precore (PC) nt 1896: The first-round PCR was performed with BCP-F7 [sense, 5'-TGC ACT TCG CTT CAC CTC TG-3' (nt 1580–1599)] and BCP-R8 [antisense, 5'-TAA GCG GGA GGA GTG CGA AT-3' (nt 2295–2276)] primers, and the second-round PCR with BCP-F5 [sense, 5'-GCA TGG AAA CCA CCG TGA AC-3' (nt 1606–1625)] and BCP-R6 [antisense, 5'-ATA CAG AGC AGA GGC GGT AT-3' (nt 2014–1995)] primers. All samples were initially denatured at 95°C for 4 min. Thirty-five cycles of amplification were set as follows: denaturation for 1 min at 94°C, annealing of primers for 2 min at 55°C, and extension for 3 min at 72°C with an additional 7 min for extension. Then 1 μl of the first-round PCR product was transferred to the second-round PCR reaction. Other conditions for the second-round PCR were the same as the first-round PCR, except that the second-round PCR primers were used instead of the first-round PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Perkin-Elmer, Chiba, Japan). To avoid false-positive results, the procedures recommended by Kwok and Higuchi [23] to prevent contamination were strictly applied to these PCR assays. No false-positive results were observed in this study.

Statistical Analysis

The χ^2 test, Fisher's exact probability test, and Mann-Whitney's U test were used to compare the background characteristics between groups. The cumulative virological and biochemical relapse rates were calculated using the Kaplan-Meier technique, differences between the curves were tested using the log-rank test. Statistical analyses of virological and biochemical relapse periods according to the mode of monotherapy (continuous and discontinuous groups) were calculated using the period from the start of lamivudine monotherapy, and those concerned with the characteristics of the discontinuous group were calculated using the period after discontinuation of the treatment. Stepwise Cox regression analysis was used to determine independent predictive factors at discontinuation of lamivudine monotherapy that contributed to early biochemical relapse after discontinuation of the treatment. We also calculated the odds ratios and 95% confidence intervals. Potential predictive factors associated with early biochemical relapse included the following ten variables at discontinuation of treatment: sex, age, histological stage, HBV genotype, levels of HBV DNA, HBeAg, pattern of BCP and PC, presence of YMDD motif mutant, and duration of lamivudine therapy. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. Variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) on univariate analysis were tested by multivariate Cox proportional hazard model to identify significant indepen-

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ient factors. Statistical comparisons were performed using the SPSS software (SPSS, Chicago, Ill., USA). All p values <0.05 by the two-tailed test were considered significant.

Results

Virological and Biochemical Relapse

Virological relapse occurred in 24.0% (18 of 75 patients) of patients of the continuous group and 84.0% (21 of 25) of the discontinuous group. The cumulative virological relapse rates of the continuous and discontinuous group were 12.3 and 54.1% at the end of one year after the commencement of lamivudine monotherapy; 26.0 and 70.8% at 2 years; and 30.1 and 87.8% at 3 years, respectively. Virological relapse in the discontinuous group emerged significantly earlier than the continuous group ($p < 0.0001$; log-rank test) (fig. 1A).

Biochemical relapse occurred in 22.7% (17 of 75 patients) of patients of the continuous group and 68.0% (17 of 25 patients) of the discontinuous group. The cumulative biochemical relapse rates of the continuous and discontinuous group were 4.27 and 32.2% at the end of one year after commencement of lamivudine monotherapy; 26.5 and 61.9% at 2 years; and 33.9 and 66.7% at 3 years, respectively. Biochemical relapse in the discontinuous group emerged significantly earlier than in the continuous group ($p = 0.0011$; log-rank test) (fig. 1B).

YMDD mutants were not detected in any of the pretreatment serum samples. Emergence of YMDD motif mutant was noted in 29.3% (22 of 75 patients) of patients of the continuous group and 12.0% (3 of 25 patients) of the discontinuous group. In the continuous group, all of 18 virological relapsers showed DNA breakthrough associated with the emergence of YMDD motif mutant, and all of 17 biochemical relapsers showed breakthrough hepatitis associated with DNA breakthrough.

ALT and Bilirubin Levels after Biochemical Relapse or Emergence of YMDD Motif Mutant

The peak levels of serum ALT and bilirubin after biochemical relapse, and the ratios of peak levels to pretreatment were not significantly different between continuation or discontinuation groups (table 3). Likewise, the peak levels of serum ALT and bilirubin after the emergence of YMDD motif mutant, and the ratios of peak levels to pretreatment were also not significantly different between the two groups (table 3).

Factors Associated with Early Biochemical Relapse after Discontinuation of Lamivudine Monotherapy

The cumulative biochemical relapse rates of the discontinuous group were 48.0, 64.8, 69.2, and 69.2% at the end of 0.5, 1, 2, and 3 years after discontinuation of lamivudine monotherapy, respectively. Potential predictive factors associated with early biochemical relapse after discontinuation of treatment were explored in 25 patients of the discontinuation group. In univariate analyses, the following six factors tended to or significantly influenced the early biochemical relapse: HBeAg ($p = 0.0048$), levels of HBV DNA ($p = 0.039$), pattern of BCP ($p = 0.026$), pattern of PC ($p = 0.033$), age ($p = 0.083$), and liver cirrhosis ($p = 0.096$). In multivariate analysis using these factors, HBeAg ($p = 0.0035$), liver cirrhosis ($p = 0.0052$), and pattern of BCP ($p = 0.015$) were independent significant predictors of early biochemical relapse after discontinuation of the treatment (table 4). The odds ratio of liver cirrhosis was 16.1 compared with the absence of cirrhosis. The odds ratio of HBeAg-positive was 5.61 compared with HBeAg-negative. The odds ratio of detectable BCP mutant virus was 3.93 compared with undetectable BCP mutant virus.

Retreatment for Biochemical Relapse after Discontinuation of Lamivudine Monotherapy

Eight of 17 patients, who showed relapse after the termination of the treatment, received another course of lamivudine monotherapy at the same dose after a median stop (no treatment) period of 0.61 years (range, 0.15–1.8 years). The median period of retreatment was 1.1 years (range, 0.14–2.7 years). Six of these patients were HBeAg-positive, and the remaining 2 were HBeAg-negative at the commencement of retreatment. Five of 8 (62.5%) patients successfully showed normalization of ALT level and disappearance of HBV-DNA after retreatment; of whom 2 were HBeAg-negative (100%) and 3 were HBeAg-positive (50%). The other 3 patients, who did not show normalization of ALT, were HBeAg-positive, and especially 2 patients showed HBeAg reversion. Furthermore, in 2 of the latter 3 nonresponders, lamivudine therapy was terminated following the emergence of YMDD motif mutant, and both developed severe biochemical relapse (a rise in ALT level to ≥ 300 IU/l, accompanied by the elevation of total bilirubin level to ≥ 2.0 mg/dl) during retreatment. In particular, one of them developed severe relapse despite HBeAg seronegative conversion and was HBV DNA undetectable for one year (fig. 2). In summary, 5 of 6 patients (83.3%) without YMDD motif mutant at discontinuation could achieve ALT normalization again with retreatment, but 2 of 2 patients with YMDD motif mutant developed severe biochemical relapse during retreatment. Hence, retreatment with lamivudine monotherapy was effective, but tended to be not very effective

for HBeAg-positive patients retreated after the emergence of YMDD motif mutant.

Discussion

Previous studies reported that the estimated half-life of hepatocytes infected with HBV was 10–100 days, suggesting that prolonged administration of lamivudine for a period longer than one year might be needed to clear HBV in the liver by turning over most of cccDNA-containing hepatocytes [24, 25]. However, a recent report by Ryu et al. [26] showed that HBV DNA and HBeAg reappeared in 31 and 16% of their patients, respectively at 2 years after the termination of lamivudine, even when HBV DNA and HBeAg had been persistently negative for 2 years or more. Based on these findings, they suggested that long-term additional administration of lamivudine might enhance the durability of lamivudine-induced HBeAg seroconversion [26]. Our results of the discontinuous group also indicated higher cumulative biochemical relapse rates of 64.8 and 69.2% at 1 and 2 years after discontinuation, similar to the Korean report (relapse rates, 37.5 and 49.2% at 1 and 2 years) [27], although this might be due to the criteria used for the definition of the discontinuous group, regardless of HBeAg seroconversion and inclusion of subjects who were HBeAg-negative at the start of the treatment.

With regard to long-term treatment, while continued disease suppression, or even HBeAg seroconversion, still occurred in some patients, in others, breakthrough hepatitis associated with the appearance of YMDD mutant may occur. Severe breakthrough hepatitis has been reported despite the continuation of lamivudine [28–32], even though previous studies showed that YMDD mutants are less replication-competent compared with the wild-type, and are associated with lower HBV DNA levels compared with pretreatment HBV DNA levels [4, 5, 33–37]. We have recently reported that 3-year lamivudine therapy induced histopathological improvement regardless of the appearance of YMDD mutants, associated with DNA breakthrough and breakthrough hepatitis, and suggested the benefit of long-term treatment [38].

In our study based on patients matched for age, sex, and observation period, the cumulative virological and biochemical relapse rates were compared according to the continuation or not of lamivudine monotherapy. Our results showed that the relapse rates in the discontinuous group emerged significantly earlier than the continuous group. Furthermore, the peak levels of serum ALT and bilirubin and the ratios of peak to pretreatment levels were not significantly different between the continuation and discontinuation groups, regardless of the emergence of YMDD motif mutant. To our knowledge, this is the first report based on matched patients' backgrounds that

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ompares the virological and biochemical relapse rates ccording to continuation or discontinuation of lamivudine monotherapy.

One limitation of our study is the small number of patients, the use of various treatment periods, and differences in the discontinuation criteria regardless of HBeAg seroconversion in the discontinuous group. Large-scale prospective studies of each group should be conducted in the future to confirm these findings.

Previous studies showed that HBeAg-positivity, old age, high pretreatment viral loads, and the presence of PC mutant at the start of the treatment might affect the biochemical relapse after treatment [39–41]. Our study based on multivariate analysis evaluated various aspects of clinicopathological characteristics at the termination of treatment, and identified HBeAg-positivity, liver cirrhosis, and detectable BCP mutant virus as independent significant determinants of early biochemical relapse. Mutations in BCP, increase viral replication and enhance disease activity [42, 43], and are also associated with HBV genotype C and a longer duration of infection (including the higher age, and more advanced liver disease) [44]. These results suggest that the presence of BCP mutant and liver cirrhosis might indicate the more active state of disease, and might be the responsible factors of an early relapse. In our study, the majority of patients of the discontinuous group were Japanese patients infected with HBV genotype C and were positive for a family history of HBV infection (namely, genotype C patients with the longer duration of infection), and thus the presence of BCP mutant together with genotype C and a longer duration of infection might explain the higher viral replication and biochemical relapse after treatment in endemic areas of HBV genotype C infection, such as Japan and Korea, where most HBV infection is considered to be transmitted vertically [27]. To our knowledge, this is the first report of early post-treatment biochemical relapse based on characteristics at discontinuation of lamivudine monotherapy. Previous reports in the United States indicated that viral suppression was maintained after the termination of treatment [45]. The discrepancy between the USA reports and our results are probably due to the differences in HBV genotypes, duration of infection, and follow-up period after the termination of treatment. Further studies of a large group of patients are required to clarify whether the patients' characteristics including HBV genotype and duration of infection affect the early virological and biochemical relapse after the termination of lamivudine monotherapy.

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Reinstitution of lamivudine monotherapy is usually effective in controlling exacerbations in patients who have not experienced breakthrough and may result in subsequent HBeAg seroconversion [39], but the benefits of retreatment are usually transient in patients with breakthrough since YMDD mutant rapidly reappears (often within weeks) when lamivudine is resumed [46, 47] because of possible persistence of YMDD mutant over long periods after the cessation of therapy [48]. In the present study, 83.3% of patients without YMDD motif mutant at discontinuation achieved ALT normalization again with retreatment, but all (100%) patients with YMDD motif mutant developed severe biochemical relapse during retreatment. These results suggest that care should be exercised in the management of patients in whom lamivudine is first discontinued then used again, especially those who show the emergence of YMDD motif mutants.

In conclusion, the present study indicates that the discontinuation of lamivudine monotherapy for Japanese genotype C-dominant hepatitis B should be followed carefully for virological and biochemical relapses. Further prospective studies are necessary to determine the true risk of post-treatment relapse by discontinuation and breakthrough hepatitis by continuation of long-term treatment. However, it should be stated here that it would be difficult to perform such studies based on ethical grounds. Interferon therapy and new nucleotide analogs (for example, adefovir dipivoxil and entecavir) have been recently shown to be effective in patients with YMDD mutants induced by long-term lamivudine administration [49–52]. Thus, new combination therapies of antiviral drugs or alternative drugs are expected to appear in the future.

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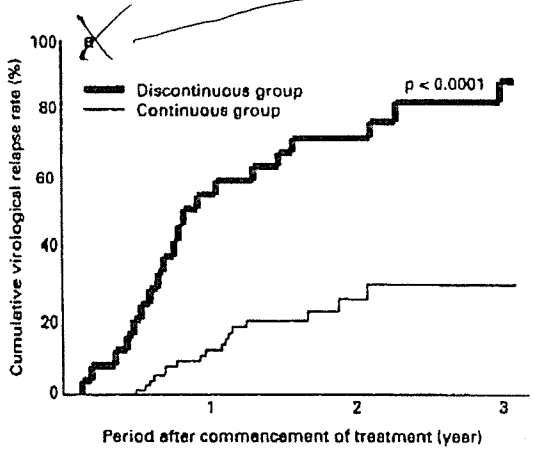

Fig. 1. a

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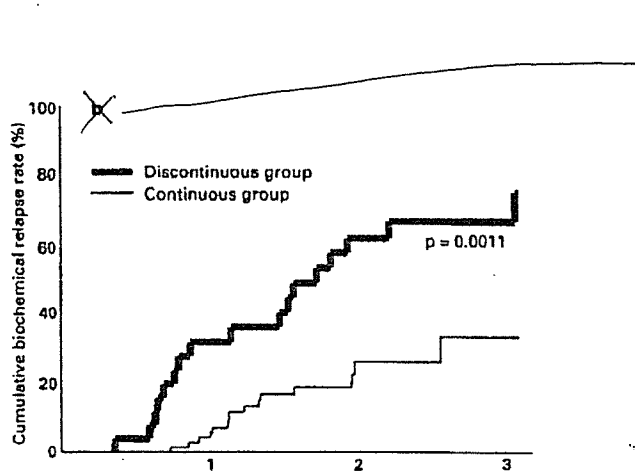

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Fig. 1. Virological and biochemical relapse rates according to the continuation or discontinuation of lamivudine monotherapy, in patients matched for age, sex, and observation period after the start of treatment. **A** Cumulative virological relapse rates after the commencement of treatment. **B** Cumulative biochemical relapse rates after the commencement of treatment. Virological and biochemical relapse in the discontinuous group emerged significantly earlier than in the continuous group.

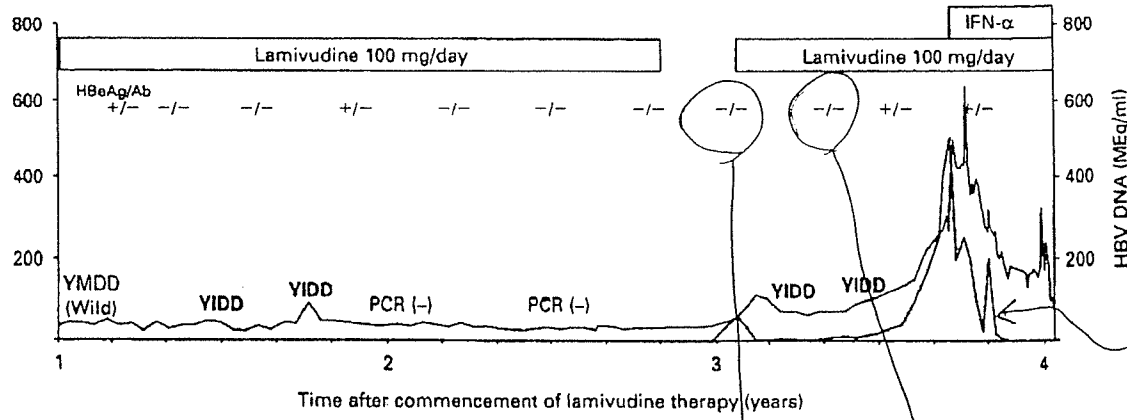

Fig 2. Clinical summary of a 34-year HBeAg-positive male patient infected with HBV-genotype C complicated with liver cirrhosis. The patient was treated with lamivudine monotherapy for 2.8 years, which resulted in HBeAg negativity and a decrease in HBV DNA to undetectable levels as measured by TMA-HPA for one year or more, despite the emergence of YMDD motif mutant (YIDD type). After the discontinuation of lamivudine, however, the patient developed severe biochemical relapse, during retreatment of lamivudine monotherapy. The case was later controlled when combination therapy of lamivudine and IFN-α was used. HBV DNA was indicated by branched DNA signal amplification technology (Chiron Corp., Emeryville, Calif., USA) to show the viral loads of higher ranges, and the results were expressed as 10^6 genomic equivalents per millilitre (MEq/ml). Thick line = HBV DNA level, thin line = ALT level.

Table 1. Clinical characteristics of enrolled patients

	Discontinuous group (n = 25)	Continuous group (n = 75)	p value
Age, years[a]	33 (19–75)	34 (20–75)	matched
Sex, male/female	19/6	57/18	matched
Period of observation[b]	2.1 (0.68–7.9)	1.8 (0.71–7.6)	matched
HBV DNA, LGE/ml[a]	7.6 (<3.7 to >8.7)	7.5 (<3.7 to >8.7)	NS
HBeAg, number of positive	20 (80.0%)	45 (60.0%)	NS
HBV genotype, number of C	22 (88.0%)	61 (81.3%)	NS
Liver cirrhosis[c]	2 (8.0%)	3 (4.0%)	NS
Family history of liver disease[d]	18 (72.0%)	59 (78.7%)	NS
T-Bil, mg/dl[a]	0.7 (0.3–20.7)	0.7 (0.3–10.5)	NS
ALT, IU/l[a]	97 (51–3,168)	150 (53–2,274)	NS
Albumin, g/dl[a]	3.9 (2.8–4.3)	3.8 (2.5–4.8)	NS
Cholinesterase, ΔpH[a]	1.0 (0.6–1.5)	1.1 (0.5–1.7)	NS
Duration of lamivudine therapy, years[a]	0.72 (0.10–5.6)	1.8 (0.71–7.6)	<0.0001

[a] Data expressed as median (range).
[b] Period of follow-up after the start of lamivudine therapy.
[c] Scoring according to the system of Desmet et al. [22].
[d] Family history of positivity for hepatitis B surface antigen including third-degree relatives.
LGE = Logarithm of genome equivalent per millilitre; T-Bil = total bilirubin; ALT = alanine transferase (normal range ≤ 50 IU/l); NS = not significant.

Table 2. Characteristics of patients at discontinuation of lamivudine monotherapy

Number	25
Sex, male/female	19/6
Age, years[a]	34 (19–75)
Number of cirrhosis	2 (8.0%)
HBV genotype, number with genotype C	22 (88.0%)
Family history of liver disease[b]	18 (72.0%)
HBeAg, number of positive	9 (36.0%)
HBV DNA, patients with <3.7 LEG/ml	18 (72.0%)
T-Bil, mg/dl[a]	0.7 (0.3–1.3)
ALT, IU/l[a]	23 (10–50)
BCP nt 1762/1764 (W/M/mi/N)	5/8/2/10
PC nt 1896 (W/M/mi/N)	10/3/2/10
Presence of YMDD motif mutant	3 (12.0%)[c]
Duration of lamivudine therapy, years[a]	0.72 (0.10–5.6)

[a] Data expressed as median (range), or number of patients.

[b] Family history of positivity for hepatitis B surface antigen including third-degree relatives.

[c] One patient was PCR-negative with serum sample at stop of treatment, but had been already detected before stop.

Abbreviations, as in table 1, BCP = Basic core promoter; PC = precore; nt = nucleotide. W = wild type (BCP; A1762G1764; PC; G1896); M = mutant type; mi = mixed type of wild and mutant virus; N = PCR-negative.

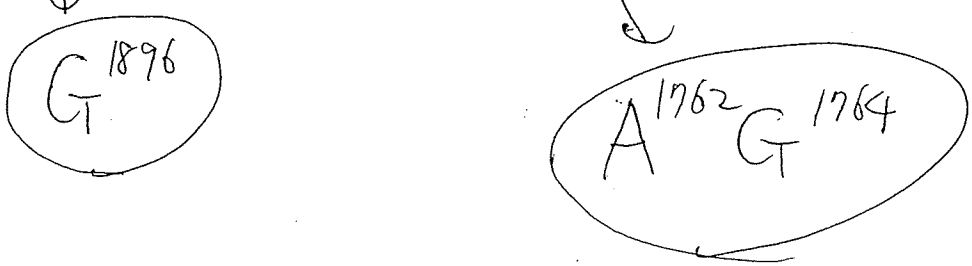

Table 3. Comparison of ALT and T-Bil levels after biochemical relapse and the emergence of YMDD motif mutant between patients who continued and those who discontinued lamivudine monotherapy

	Discontinuous group	Continuous group	p value
Biochemical relapse cases (n = 34)	17	17	~~NS~~
Peak T-Bil, mg/dl[a]	0.9 (0.5–3.8)	1.1 (0.5–4.9)	NS
Peak ALT, IU/l[a]	384 (191–1,480)	538 (67–1,736)	NS
T-Bil ratio[b]	1.5 (0.3–3.5)	2.0 (0.2–12.3)	NS
ALT ratio[b]	3.9 (1.0–18.7)	3.5 (0.1–10.8)	NS
YMDD motif mutant cases (n = 25)	3	22	~~NS~~
Peak T-Bil, mg/dl[a]	2.1 (0.7–3.8)	1.1 (0.5–4.9)	NS
Peak ALT, IU/l[a]	441 (50–638)	236 (26–1,736)	NS
T-Bil ratio[b]	1.0 (0.3–3.5)	1.8 (0.1–12.3)	NS
ALT ratio[b]	5.9 (0.6–11.2)	1.7 (0.1–10.8)	NS

[a] Data expressed as median (range).
For abbreviations, see table 1.

(b) the ratios of peak level to pretreatmen

Table 4. Predictors of early biochemical relapse after lamivudine monotherapy, determined by multivariate analysis

Factors	Category	Odds ratio (95% confidence interval)	p
Histology	1: no cirrhosis	1	~~0.0052~~
	2: cirrhosis	16.1 (2.30–113)	0.0035
HBeAg	1: negative	1	~~0.013~~
	2: positive	5.61 (1.77–17.8)	
Basic core promoter (A1762G1764)	1: undetectable mutant	1	
	2: detectable mutant	3.93 (1.31–11.8)	

Variables that achieved statistical significance ($p < 0.05$) on multivariate Cox proportional hazard model are shown.

— End —

Proof

JMV-04-745

Journal of Medical Virology 9999:1–8 (2005)

Favorable Efficacy of Long-Term Lamivudine Therapy in Patients With Chronic Hepatitis B: An 8-Year Follow-Up Study

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The long-term efficacy of lamivudine therapy in patients with hepatitis B virus (HBV) infection is still not clear. In this study, 20 non-cirrhotic Japanese patients infected with HBV received lamivudine therapy for more than 1 year and were followed for a median period of 8.5 years (range, 6.7–8.7 years). The rates of HBe antigen (HbeAg) negative, HBV-DNA undetectable, and alanine aminotransferase (ALT) normal level at the start of lamivudine were 55%, 25%, and 20% and 85%, 80%, and were 80%, respectively, at the last visit, including patients who received additional treatment. The values at the last visit tended to and were significantly higher than those at the start. The values improved at the last visit regardless of the emergence of YMDD motif mutant and continuation of lamivudine. YMDD mutant and biochemical relapse with mutant virus (breakthrough hepatitis) appeared in 65% and 45% during follow-up, respectively, but severe breakthrough hepatitis occurred in only 5%. Furthermore, 80% of patients who received additional treatment for breakthrough hepatitis, regardless of continuation of lamivudine, were ALT normal level at the last visit, in contrast to 25% untreated. HBsAg clearance occurred in two patients of the discontinuous lamivudine group with non-vertical transmission, who were relatively young. One was infected with HBV genotype C with breakthrough hepatitis and the other had no YMDD mutant and was infected with genotype D, a rare type in Japan. None developed cirrhosis or hepatocellular carcinoma (HCC) during follow-up. Our results suggest that long-term lamivudine therapy improves long-term prognosis, especially when additional treatment for breakthrough hepatitis is used. ***J. Med. Virol. 00:1–8, 2005.***

KEY WORDS: YMDD motif mutant; HBV genotype; breakthrough hepatitis; HBsAg clearance; hepatocellular carcinoma

INTRODUCTION

Lamivudine, an oral cytosine nucleoside analog clinically used for the treatment of chronic hepatitis B virus (HBV) infection, potently inhibits HBV replication by interfering with HBV reverse transcriptase activity [Doong et al., 1991; Dienstag et al., 1995; Nevens et al., 1997; Lai et al., 1998], and results in marked decrease of HBV-DNA and alanine aminotransferase (ALT) levels, seroconversion of HBe antigen (HBeAg) to anti-HBe (HBeAb), and histopathological improvement [Lai et al., 1998; Dienstag et al., 1999; Suzuki et al., 1999; Liaw et al., 2000; Schalm et al., 2000; Leung et al., 2001; Akuta et al., 2003a]. However, lamivudine-resistant HBV strains (YMDD motif mutant) have been reported in long-term lamivudine therapy, and the emergence of such mutant virus results in re-elevation of HBV-DNA (DNA breakthrough) and ALT (breakthrough hepatitis) [Tipples et al., 1996; Bartholomew et al., 1997; Lai et al., 1998; Dienstag et al., 1999; Liaw et al., 2000; Schalm et al., 2000; Leung et al., 2001; Yuen et al., 2001; Akuta et al., 2003a,b].

The optimal duration of lamivudine therapy for HBV-infected patients is still controversial for two main reasons; drug resistance and sustainability of the response to treatment. In particular, there is a need to evaluate short-term treatment with respect to post-treatment safety and the sustainability of the response

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