Low rate of YMDD motif mutations in polymerase gene of hepatitis B virus in chronically infected patients not treated with lamivudine

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Introduction

Lamivudine is used for the treatment of chronic hepatitis B (CH-B), and exhibits excellent antiviral activity. However, longterm administration increases the likelihood of the emergence of resistant viruses, with an accompanying relapse of hepatitis. However, recent studies have reported lamivudine-resistant viruses in patients with CH-B before such treatment. The aim of this study was to investigate whether YMDD mutants occur in nature. Methods. The existence of lamivudine-resistant viruses was examined in 20 asymptomatic carriers of hepatitis B virus (ASC), 10 patients who lost hepatitis B surface antigen (HBsAg) during follow-up and in 20 lamivudine-treated patients with and without breakthrough hepatitis. Both polymerase chain reaction (PCR) restriction fragment length polymorphism and SMITEST hepatitis B virus (HBV)-YMDD mutation detection methods were used to detect resistant viruses. Results. No YMDD mutants were detected in the sera of the 20 ASC at the initial and final medical examinations, nor were YMDD mutants detected in sera collected at the initial medical examination, about 6 months before, or immediately after the loss of HBsAg in the 10 patients. In the 20 patients treated with lamivudine, YMDD mutants were not detected in any of them before treatment, whereas mutants were detected in the sera of 10 patients during treatment. Conclusions. Our results suggest that lamivudine-resistant YMDD mutant viruses were present in a few patients with HBV infection who before they have been treated with lamivudine.

Key words: hepatitis B virus, lamivudine, YMDD mutant
Subjects

The study subjects were 50 patients with hepatitis B who were being followed-up at Toranomon Hospital. Three groups were investigated: (1) group A, 20 ASC who showed normal transaminase levels for 10 or more years (aged 25 to 77 years; median, 47.5 years), comprising 8 men and 12 women. Hepatitis B e antigen (HBeAg) was positive in 1 patient and negative in 19 patients, as determined by radioimmunoassay (RIA) or enzyme immunoassay (EIA). None of these patients had been treated with antiviral agents. (2) Group B, 10 patients with hepatitis B who had lost HBsAg during the follow-up (aged 31 to 51 years; median, 43 years), comprising 8 men and 2 women. At the initial medical examination at Toranomon Hospital, HBeAg was positive in 1 patient and negative in 9 patients. The HBeAg-positive patient became antigen-negative during the follow-up. (3) Group C, 20 patients who were treated with lamivudine at Toranomon Hospital, 10 of whom had breakthrough hepatitis and the other 10 who did not. They were aged 26 to 56 years (median, 43 years) and comprised 18 men and 2 women. The patients were treated with lamivudine for 25 to 290 weeks (median, 108.5 weeks), and 7 of the 10 patients who had breakthrough hepatitis were concomitantly treated with interferon (IFN). In patients in group A, serum samples were collected at the initial medical examination and at the final examination during follow-up. In patients in group B, the samples were collected at the initial medical examination, and about 6 months before, and immediately after the loss of HBsAg. In patients in group C who had breakthrough, the samples were collected before lamivudine treatment and at breakthrough (before IFN treatment in IFN-treated patients) and in patients in group C who did not have breakthrough, the samples were collected before lamivudine treatment and at the final examination at the end of lamivudine treatment.

Methods

Detection of YMDD mutant viruses

HBV-DNA was extracted from 100 µl of serum by using SMITEST EX-R&D (Genome Science, Tokyo, Japan). YMDD mutant viruses were detected by a combination of PCR-ELISA and a minisequence method (PCR-ELMA method; SMITEST HBV-YMDD mutation detection kit; Genome Science) and by a PCR-RFLP method.

HBV DNA levels were measured by transcription-mediated amplification and hybridization protection assay (TMA-HPA) (Chugai Diagnostics Science, Tokyo, Japan) in patients treated with lamivudine, at baseline and 3 months after commencement of the therapy. The lower and higher limits of detection of this assay are 3.7 and 8.7 log genome equivalents per milliliter (LGE/ml), respectively.

Results

Serum samples obtained from ASC at the initial medical examination were HBV-DNA negative in 2 of the 20 patients and positive (YMDD) in the remaining 18 patients by PCR-RFLP, whereas the serum samples were HBV-DNA-positive (YMDD) in all patients by PCR-ELMA. Serum samples obtained from ASC at the final examination were HBV-DNA-negative in 7 patients and positive (YMDD) in the remaining 13 patients by PCR-RFLP, whereas they were HBV-DNA-negative in 6 patients and positive (YMDD) in 14 patients by PCR-ELMA. No YMDD mutant virus was detected by either method (Table 1).

Sera obtained at the initial medical examination from the patients who had lost HBsAg were HBV DNA-positive (YMDD) in all patients by PCR-RFLP, but were HBV DNA-positive (YMDD) in five of the ten patients and negative in the remaining five patients by PCR-ELMA. Serum samples obtained approximately 6 months before the loss of HBsAg were HBV DNA-negative in four patients and positive (YMDD) in six patients by both PCR-RFLP and PCR-ELMA. The serum samples obtained immediately after the loss of HBsAg were HBV-DNA-negative in four patients and positive (YMDD) in six patients by PCR-RFLP, but they were negative in nine patients and positive (YMDD) in 1 patient by PCR-ELMA. No YMDD mutant virus was detected by either method (Table 2).

Sera obtained from lamivudine-treated patients before such treatment were HBV DNA-positive in all patients by the PCR-RFLP method as well as by PCR-ELMA. Sera obtained during lamivudine treatment were HBV DNA-negative in ten patients by PCR-RFLP, whereas YIDD and/or YVDD was detected in the sera of the remaining ten patients by this method. By PCR-ELMA, the sera were HBV DNA-negative in three patients and positive (YMDD) in seven patients, whereas YIDD and/or YVDD was detected in the sera of ten patients by this method. The YMDD mutant viruses were detected in the same ten patients by both methods, and all of these patients had breakthrough (Table 3). On the other hand, HBV DNA levels were decreased after 3 months of lamivudine treatment in all patients (Fig. 1).