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Dynamic changes of hepatitis B virus polymerase gene including YMDD motif in lamivudine-treated patients with chronic hepatitis B

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Summary

The mutation of YMDD motif of hepatitis B virus (HBV) polymerase gene is the most frequent cause in HBV resistant to lamivudine. The aim of the study was to investigate variation features of HBV polymerase gene in chronic hepatitis B (CHB) patients before and after lamivudine treatment. From the serum samples of five CHB patients before and after 12 months of lamivudine treatment, HBV polymerase gene was amplificated and positive DNA fragments were cloned into JM105 competent cell. Twenty positive clones of every sample were checked with mismatched polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and YMDD variants were sequenced. Among five patients after 12 months of lamivudine treatment, M552I mutations in two patients with HBV DNA rebounding and D553G mutation in one non-responder were detected except two patients with negative HBV DNA consecutively. In summary, D553G mutation is probably one of the reasons that caused non-responders during lamivudine treatment. The mutations of YMDD motif occurred after lamivudine treatment are caused by the induction of lamivudine.
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Introduction

Hepatitis B virus (HBV) infection is a major health problem leading to around one million deaths annually worldwide. It causes widely clinical manifestations ranged from asymptomatic carriers to severe chronic liver disease, including those with cirrhosis and hepatocellular carcinoma. In China, HBV infection rate is 10% or so and there are approximately 100 million HBV carriers. Antiviral treatment is central to those patients with chronic hepatitis B (CHB). Although interferon therapy has

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benefited some patients with CHB for 20 years, the overall response rate is less than 40% (Hoofnagle and Bisceglie, 1997).

The approval of lamivudine has revolutionized the treatment of CHB. Lamivudine is an orally administered nucleoside analog with an excellent safety profile. It can markedly reduce serum HBV DNA levels and normalize alanine aminotransferase (ALT) levels associated with improvement in liver necroinflammatory activity (Lai et al., 1998). However, more and more questions are found during anti-HBV treatment. Of these questions, the greatest drawback is the appearance of drug-resistant HBV mutants. The most common mutation affects the highly conserved the tyrosine–metheonine–aspartate–aspartate (YMDD) motif in the catalytic domain of the HBV reverse transcriptase (P gene) (Dienstag et al., 1999), resulting in a methionine to valine or isoleucine substitution at codon 552 (M552V or M552I). With the emergence of YMDD mutation, virological and biochemical breakthrough may occur. Furthermore, fatal hepatic failure after the emergence of the YMDD mutants during lamivudine therapy has been reported (Wang et al., 2002). On the other hand, a part of patients with CHB have no response to the antiviral therapy. Most studies about this have focused on the search for predictors of response to lamivudine. By far the reason why the drug-resistant mutants may occur is controversial. What's more, there is no consensus on the mechanism of non-response in the patients received lamivudine therapy.

In this study, we followed-up five patients with CHB and examined dynamic changes of HBV polymerase gene, and grounded for investigating the way predicting the effect of lamivudine.

Materials and methods

Patients

Five consecutive patients with CHB were studied. All of them were men (age range 30–49 years) and have received oral lamivudine therapy (100 mg, qd) for at least 1 year at the affiliated hospital of Xuzhou Medical College. One of them was a carrier with HBV and the others were typically CHB patients whose serum alanine transaminase (ALT) levels are above 2 times upper limit of normal (ULN). All the patients were hepatitis B s antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B c antibody (anti-HBc) and positive HBV DNA before treatment (Table 1). The diagnosis of CHB was based on standard diagnostic criteria, as formulated by the Fifth China Infectious Disease and Parasitology Conference in 2000.They had no complications of other organs and serologically negative for antibodies to HAV, HCV and HGV. Serial sera pretreatment and at 3 month intervals during treatment were tested for liver biochemistry, HBV markers and HBV DNA.

Serology assays

Serology markers for HBV, for hepatitis C (anti-HCV), for hepatitis D (anti-HDV), and for hepatitis G (anti-HGV) were tested by commercially available enzyme immunoassay kits.

HBV DNA assays

Serum HBV DNA was detected using fluorescence quantitative-PCR, which has a detection limit of 10^3 copies/mL.

DNA extraction and PCR amplification

DNA was extracted from 100 μL of serum. Serum was incubated for protein digestion in 100 μL of lysis buffer (0.1 mol/L Tris–HCl, pH 8.0, 0.1 mol/L ethylenediaminetetraacetic acid, 5% sodium dodecyl sulfate) containing 2 μL of proteinase K at 25 mg/mL for 4 h. DNA was extracted with phenol/chloroform/isoamyl alcohol, precipitated by ethanol for 1 h at −40 °C and resuspended in ddH_2O. A final volume of 50 μL containing 50 mmol/L Tris–HCl, 40 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 250 mmol/L of each deoxynucleotide, 2.5 u of pfu DNA polymerase and 20 pmol of primer P1 and P2 performed amplification of P gene by PCR. After a denaturation step at 94 °C for 5 min, the reaction

Table 1. Baseline data of the five patients with chronic hepatitis B in this research

No. of patients	Age (years)	Sex	ALT (u/L)	HbeAg	log HBV DNA	YMDD mutations
1	34	M	120	+	6+0.16	—
2	37	M	86	+	7+0.61	—
3	42	M	117	+	8+0.75	—
4	30	M	22	+	8+0.51	—
5	40	M	215	+	9+0.02	—

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