

Dynamic changes of HBV DNA in serum and peripheral blood mononuclear cells of chronic hepatitis patients after lamivudine treatment

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CONCLUSION: Lamivudine has remarkable inhibitory effects on HBV replication both in serum and in PBMCs. The inhibitory effect on HBV DNA in PBMCs is weaker than that in serum.

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Key words: Lamivudine; Hepatitis B virus; DNA; Peripheral blood mononuclear cells

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Abstract

AIM: To study the dynamic changes of hepatitis B virus (HBV) DNA in serum and peripheral blood mononuclear cells (PBMCs) of patients after lamivudine therapy.

METHODS: A total of 72 patients with chronic HBV infection were included in this study. All patients were confirmed to have the following conditions: above 16 years of age, elevated serum alanine aminotransferase (ALT), positive hepatitis B e antigen (HBeAg), positive HBV DNA in serum and PBMCs, negative antibodies against HAV, HCV, HDV, HEV. Other possible causes of chronic liver damages, such as drugs, alcohol and autoimmune diseases were excluded. Seventy-two cases were randomly divided into lamivudine treatment group ($n = 42$) and control group ($n = 30$). HBV DNA was detected both in serum and in PBMCs by fluorescence quantitative polymerase chain reaction (PCR), during and after lamivudine treatment.

RESULTS: In the treatment group, HBV DNA became negative both in serum and in PBMC, of 38 and 25 out of 42 cases respectively during the 48 wk of lamivudine treatment, the negative rate was 90.5% and 59.5% respectively. In the control group, the negative rate was 23.3% and 16.7% respectively. It was statistically significant at 12, 24 and 48 wk as compared with the control group ($P < 0.005$). The average conversion period of HBV DNA was 6 wk (2-8 wk) in serum and 16 wk (8-24 wk) in PBMC.

INTRODUCTION

Lamivudine (LAM) is a new antiviral agent against hepatitis B virus (HBV) infection. It has been shown that lamivudine therapy can rapidly reduce HBV DNA levels in serum and improve liver histology^[1-3]. However, the dynamic changes of HBV DNAs in serum and peripheral mononuclear cells (PBMCs) in patients after lamivudine therapy are not clear, especially in PBMCs.

PBMCs contain various kinds of active immune cells. The activities of immune cells affect directly the results and efficacy of antiviral therapy^[4,5]. Few studies are available on the dynamic changes of HBV DNA in serum and PBMCs. The aim of this study was to investigate the inhibition of HBV DNA in serum and PBMCs in patients after LAM therapy.

MATERIALS AND METHODS

Patients and materials

A total of seventy-two patients with chronic hepatitis B were from Out-patient and In-patient Departments of our hospital during February 2003-February 2004. There were 50 males and 22 females aged 18-60 years (average 32.4 years). Diagnosis of hepatitis B was made according to the revised standard of the diagnosis established at the Tenth National Symposium on Viral Hepatitis in Xi'an in

Table 1 Change of HBV DNA in serum and PBMC of chronic hepatitis B patients after lamivudine treatment *n* (%)

Group	HBVDNA in serum				HBVDNA in PBMC			
	4 wk	12 wk	24 wk	48 wk	4 wk	12 wk	24 wk	48 wk
Lamivudine Treatment 42	9 (21.1)	26 (61.9) ^a	34 (80.9) ^a	38 (90.5) ^a	4 (9.5)	15 (35.7) ^a	20 (47.6) ^a	25 (59.5) ^a
Routine Treatment 30	2 (6.7)	3 (10.0)	5 (16.7)	7 (23.3)	1 (3.3)	2 (6.7)	4 (13.3)	5 (16.7)

^a*P* < 0.005 vs control group.

2000^[6]. All patients were confirmed to have the following conditions: above 16 years of age, normal or abnormal ALT, positive HbsAg and HBeAg in serum, negative anti-HCV, anti-HDV and anti-HEV in serum, positive HBV DNA in serum and PBMCs.

Methods

Seventy-two patients with chronic hepatitis B were randomly divided into two groups: lamivudine treatment group (*n* = 42) receiving 100 mg lamivudine daily for 48 wk, control group (*n* = 30) receiving routine medication with vitamin C and inosine, *etc.* All samples were prepared and stored at -20°C for further examination. HBV DNA in serum and PBMCs was detected after 4, 12, 24 and 48 wk of treatment.

Quantitative determination of HBA DNA in serum and PBMCs

HBV DNA in serum was detected by fluorescence quantitative PCR assay, strictly according to the manufacturer's instructions (Da An Gene Institute, Shenzhen, No: 20030041).

Lymphocyte separation medium (Second Reagent Factory of Shanghai, No. 200304) was used to separate PBMCs. After isolation, the cells were washed three times with PBS containing 1% brine. The cells were diluted to 1×10^6 /mL with RPMI before detection.

Cellular DNA was extracted according to NaI method. Briefly, 100 μ L 1×10^6 /mL cell suspension and 200 μ L NaI were well mixed, inverted for 20 s, then mixed with 400 μ L chloroform/iso-amy alcohol (24:1) and spun by centrifugation at 10 000 r/min for 12 min. Three hundred microlitre supernatant was mixed with 200 μ L pure dimethyl carbinol, spun by centrifugation for 12 min at 14 000 r/min. Supernatant was collected and mixed with 1 mL 70% ethanol, the DNA was precipitated at -20°C.

HBV DNA in PBMCs was also detected by fluorescence quantitative PCR assay. The reaction conditions were: pre-denaturation at 93°C for 3 min, followed by 40 cycles of denaturation at 93°C for 45 s, extension at 55°C for 60 s and a final extension at 93°C for 30 s, at 55°C for 60 s. The reference graph was drawn according to standard content by a computer. The amount of HBV DNA was calculated. During PCR, a strict control was performed. All results were negative.

Statistical analysis

Statistical analysis was carried out by chi square test and *t*-test. *P* < 0.05 was considered statistically significant.

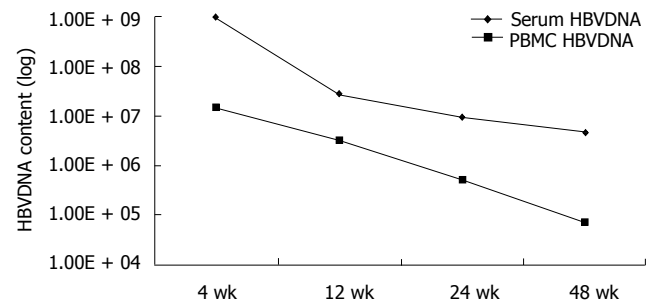


Figure 1 Content of HBV DNA in serum and PBMC of chronic hepatitis B patients after lamivudine treatment.

RESULTS

The content of HBV DNA in serum and PBMC was decreased after lamivudine treatment (Table 1, Figure 1).

DISCUSSION

Lamivudine is a HBV polymerase inhibitor and acts on HBV replication both *in vitro* and *in vivo*. It has been shown that lamivudine can suppress HBV replication, decrease transaminase levels and improve liver histology^[7-10]. However, the main goal of treatment in chronic hepatitis B is to eliminate or persistently inhibit the replication of HBV. After long-term application of lamivudine, antiviral resistance may occur due to HBV polymerase mutation, manifested as increased HBV DNA and serum transaminase, suggesting that the content of HBV DNA can be used to evaluate the efficacy of antiviral therapy. Lamivudine is not able to eliminate HBV ccc DNA in hepatocytes^[11], which is the main reason for HBV DNA rebound after withdrawal. Studies have shown that patients with hepatitis B usually accompany disorder of immune function caused by immunological injury^[12-25]. PBMCs contain various immune active cells, and play a significant role in immune responses during antiviral therapy. It has been shown that HBV infection may exist in PBMCs^[4,26,27]. When PBMCs are infected with HBV, they not only affect the function of immunity, but also weaken the efficacy of antiviral drugs. Therefore, the dynamic change of HBV DNA in PBMCs may be a reliable index for curative antiviral efficacy.

In our study, lamivudine had remarkable inhibitory effects on HBV DNA both in serum and in PBMCs. When the treatment time was prolonged, the negative rate of HBV DNA in serum and PBMCs was gradually increased. The eliminate effect of lamivudine on HBV DNA in

PBMCs was lower than that in serum. The negative rate of HBV DNA in serum was 90.48%, but only 59.52% in PBMCs after lamivudine treatment. HBV DNA was positive in 17 cases of chronic hepatitis B after 48 wk of treatment, which may be associated with the fact that mutations occurred in HBV after LAM therapy^[28], HBV DNA in PBMCs interferes with the immune activity of cells after HBV integrates with PBMCs, and decreases the contents of Ig, C₃, TNF and activity of NK cells as well as the ratio of CD4⁺/CD8⁺^[4,5,29]. Therefore, the function of cell-mediated immunity and humoral immunity are reduced and affect the curative effect of lamivudine.

In conclusion, HBV DNA exists in PBMCs even after 48 wk of lamivudine treatment. The presence of HBV DNA in PBMCs may infect hepatocytes again and cause the relapse of hepatitis.

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