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ORIGINAL ARTICLE

The effect of peginterferon alpha-2a vs. interferon alpha-2a on intrahepatic covalently closed circular DNA in HBeAg-positive chronic hepatitis B patients

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Summary

Background and objective: The covalently closed circular DNA (cccDNA), as the template of HBV transcription, plays a key role in the virus infection. The present study aimed to compare the effect of pegylated interferon (IFN)-α-2a with that of conventional IFN-α-2a on intrahepatic covalently closed circular (ccc)DNA in patients with chronic hepatitis B.

Methods: Seventy-six HBeAg-positive chronic hepatitis B patients were randomly divided into two groups ($n = 38$): group A was treated with interferon alpha-2a (IFN-α-2a) and group B was treated with peginterferon alpha-2a (peg IFN-α-2a). The intrahepatic level of cccDNA and its detection rate, levels of hepatitis B virus (HBV) DNA in liver and serum, histologic inflammation and some biochemistry parameters (alanine aminotransferase, aspartate aminotransferase and total bilirubin levels) were measured.

Results: The outcome of 48 weeks therapy showed that the mean level of intrahepatic HBV cccDNA level and its detection rate, the levels of HBV DNA and the histology and biochemistry parameters were significantly decreased following therapy in two groups ($P < 0.05$). While, the reductions in the group treated with peg IFN-α-2a were greater ($P < 0.05$).

Conclusions: It may be concluded that the ability of the peg IFN-α-2a to clear and suppress cccDNA and HBV DNA was superior compared with that of conventional IFN-α-2a. Furthermore, the effects of peg IFN-α-2a on histology and biochemistry parameters were also more obvious than conventional IFN-α-2a.

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Introduction

Hepatitis B virus (HBV) infection, one of the most common viral infections, may cause acute and chronic liver diseases and remains a major health problem worldwide. Many of the patients with chronic hepatitis B have a high risk of developing cirrhosis or hepatocellular carcinoma (HCC) [1]. The factors associated with disease progression to cirrhosis involve prolonged replicative phase, elevated HBV DNA levels, infection with hepatitis C, hepatitis D and concurrent alcohol use [2]. HBV is a member of the *Hepadnaviridae* family, which includes small enveloped DNA viruses. The ultimate goal of treatment is therefore to prevent these complications by achieving adequate viral suppression. The covalently closed circular DNA (cccDNA), as the template of HBV transcription, plays a key role in the life cycle of the virus and permits the persistence of infection.

Current antiviral therapies for chronic hepatitis B involve the use of nucleoside analogs and interferon alpha (IFN-α) [3,4]. However, conventional interferon alpha requires subcutaneous administration daily or three times weekly and is associated with frequent adverse events [5]. In addition, there is more active host immune response directed towards clearance of the infection with raised ALT levels [6]. These drawbacks of conventional interferon alpha therapy have led to the development of pegylated interferon alpha (peg IFN-α). Pegylation of interferon alpha-2a (IFN-α-2a) could enhance the pharmacokinetic properties of unmodified interferon alpha and enable once a week dosing. Pegylated interferon alpha-2a (peg IFN-α-2a) as a first-line therapy for chronic HBV is also because of its efficacy in inducing off therapy sustained disease remission [7].

The present study aimed to compare the effects of peg IFN-α-2a with those of conventional IFN-α-2a on the intrahepatic levels of cccDNA in HBeAg-positive chronic hepatitis B patients. The HBV DNA and biochemical parameters were also observed.

Materials and methods

Subjects

After approval of the study protocol by our institutional Medical Ethics Committee, written informed consent was obtained from each patient. Between August 2010 and August 2011, seventy-six patients with HBeAg-positive chronic hepatitis B were recruited, including 59 males and 17 females aged 29—47 (mean 38.1) years. All of the patients were from the Fifth Hospital of Shijiazhuang. The subject inclusion criteria were as follows: positive for HBsAg and HBeAg, serum HBV DNA $\geq 1 \times 10^3$ copies/mL, serum alanine aminotransferase (ALT) levels at least two-fold higher than the normal range (normal range = 0.667 nkat/L), without human immunodeficiency virus (HIV) infection and viral hepatitis A, C, delta or E, without alcoholism, pregnancy, cirrhosis, chronic renal failure and concurrent autoimmune disease, with the normal routine blood. Patients treated with antiviral agent or other antiviral therapies for 6 months prior to enrolment in this study were excluded. Patients were randomly allocated to 2 groups, group A (treatment with interferon alpha-2a) and group B (treatment with peginterferon alpha-2a) with 38 patients in each group. There was no significant difference in the age, gender. Group A received 3 million units of IFN-α-2a every other day with intramuscular injection. Group B received 180 μg of peg IFN-α-2a 1 time per week with subcutaneous injection. The courses were 48 weeks long. Presence of serum HBV DNA levels $\leq 1 \times 10^3$ and normalization of serum ALT levels were assessed as treatment response.

Measurement of HBV markers and biochemical tests

Blood samples were obtained before and immediately after treatment. Liver biochemistry and HBV marker test (using commercial assays, Abbott Laboratories, USA) were performed on these samples.

Liver biopsy specimens were collected by needle liver biopsies (0.5—1.5 cm) before and after treatment. The tissue was washed several times in cold phosphate-buffered saline (PBS) and stored at $-70\,^{\circ}$C.

Measurement of HBV cccDNA

DNA extraction was conducted as described previously [8]. Briefly, before cccDNA amplification, aliquots of each DNA were treated with Plasmid-safe ATP-dependent DNase (Epicenter Technology, Madison, WI). HBV cccDNA was then tested by cross-double-gap real-time PCR. The primers were synthesized for amplifying cccDNA according to the published consensus sequence. Sequences of the primers and the probe are as follows:

- 5′-CGACCACGGGGCGCACCTCTCTTTAC-3′;
- 5′-CAAGGCACAGCTTGGAGGCTTGAACAG-3′ and;
- 5′-TCTCCTCCCCAGCTCCTCCCAG-3′ (Taq Man probe).

PCR was performed at 94 °C for 2 min, followed by 45 cycles at 94 °C for 20 s, at 60 °C for 1 min. The LightCycler system (Roche Diagnostics, Mannheim, Germany) was used in real-time PCR quantification of cccDNA. We use a series of four dilutions of a single copy HBV genome containing plasmid to achieve final concentrations of approximately 10^2—10^5 copies/μL for standard curve calibration. Reagent control, negative controls, and standards and positive control were added with the tested samples in each PCR run. Quantifying the amount of β-globin was used to standardize the extracted DNA in terms of copies per genome equivalent. Quantification of β-globin was performed by real-time PCR using a commercially available kit for use in the LightCycler system (Roche DNA control kit; Roche Diagnostics).

Serum and intrahepatic HBV DNA assays

Total DNA was extracted from liver tissue with the Qiaamp DNA tissue mini DNA kit (Qiagen Inc., Germany) according to the instructions of the manufacturer. Serum and intrahepatic HBV DNA were measured quantitatively by real-time polymerase chain reaction (PCR) (Model 5700, ABI Company, USA) [9]. The range of HBV DNA detection was from 10^2 to