The Change of Quantitative of HBeAg Can Predict the Efficacy of Peg-IFN- α 2a in HBeAg-positive CHB Patients

Yong-jian Ji¹, Wan-hua Ren², Fei-fei Li², Jian-ting Fang², Xi-zhen Sun² and Cheng-yong Qin²

Objective To investigate the quantitation of hepatitis B e antigen (HBeAg) at week 24 in predicting the efficacy of pegylated-interferon alfa-2a (Peg-IFN- α 2a) in HBeAg-positive chronic hepatitis B (CHB) patients at week 48 and to find a useful predictor for treatment efficacy and investigate individualized treatment of antiviral therapy.

Methods Ninety-six HBeAg-positive CHB patients with detectable HBeAg who were treated with Peg-IFN- α 2a were enrolled in this trial. They were categorized into 3 groups according to the changes of HBeAg in week 24: HBeAg decline $> 2 \log_{10}$ group (group A), HBeAg decline between $1 \log_{10} - 2 \log_{10}$ (group B), HBeAg decline $< 1 \log_{10}$ group (group C), and group C was randomly distributed into C1 and C2. The patients in group A, group B, and group C1 continued the original therapy and the patients in group C2 were given lamivudine plus Peg-IFN- α 2a for 24 weeks. At week 48, the treatment efficacy and hepatitis B virus covalently closed circular DNA (HBV cccDNA) in liver biopsies were analyzed.

Results At week 48, mean reduction of serum HBV DNA: group A: $5.8 \log_{10}$ copies/ml, group B: $3.8 \log_{10}$ copies/ml, group C1: $2.8 \log_{10}$ copies/ml, group C2: $5.7 \log_{10}$ copies/ml, the reduction of HBV DNA in group A was greater than groups B and C1 ($P < 0.01$), that in group C1 was greater than group C2 ($P < 0.01$), the difference between groups B and C1 had no statistical significance ($P = 0.19$). Mean reduction of HBeAg: group A: $2.7 \log_{10}$ S/CO, group B: $1.9 \log_{10}$ S/CO, group C1: $0.9 \log_{10}$ S/CO, group C2: $1.5 \log_{10}$ S/CO, the difference among groups A, B and C1 and between groups C1 and C2 were statistically significant ($P < 0.01$). At week 48, HBV DNA undetectable rate in group A, group B, group C1 and group C2 were 87.5%, 34.5%, 17.4% and 81.9%, respectively, the rate in group A was greater than groups B and C1 ($P < 0.01$), that in group C1 was greater than group C2 ($P < 0.01$). HBeAg seroconversion rate were 75.0%, 24.1%, 13.0% and 22.7%, respectively, that in group A was greater than groups B and C1 ($P < 0.01$). Group A had lower cccDNA in liver tissue than group B and group C1 ($P < 0.01$). The difference of HBV cccDNA between groups B and C1 and that between groups C1 and C2 had no statistical significance.

Conclusions HBeAg decline $> 2 \log_{10}$ at week 24 in Peg-IFN- α 2a-treated hepatitis B patients suggested a better efficacy at week 48; HBeAg decline $< 2 \log_{10}$ at week 24 suggests a worse efficacy at week 48, the combined therapy of Peg-IFN- α and lamivudine could improve the clinical responses. The change of quantitative of HBeAg at week 24 may be used as a predictor of treatment effects at week 48.

Key words: Hepatitis B, chronic; Polyethylene glycols; Interferon alfa-2a; Hepatitis B e antigen

Peg-IFN- α 2a is the first-line therapy in young HBeAg-positive patients. The advantages of Peg-IFN- α 2a therapy include a limited treatment course and a high rate of HBeAg seroconversion, the antiviral therapy in patients with

chronic hepatitis B is for the prevention of cirrhosis, hepatocellular carcinoma, and HBV-related mortality.¹ However, conceding of the individual and HBV differences of HBeAg positive CHB patients, not all the patients would have a favorable response. However, it is worthwhile to identify patients who were not sensitive to antiviral therapy, and take alternative treatments for them in time. The aims of this study were to investigate the effects of changes

¹Department of Gastroenterology, Dezhou People's Hospital, Dezhou, Shandong Province 253014, China

²Department of Infectious Diseases, Shandong University affiliated Provincial Hospital, Jinan, Shandong Province 250021, China

Correspondence: Wan-hua Ren, Email: ganbingzx@163.com

of HBeAg quantity at week 24 in predicting the efficacy of Peg-IFN- α 2a in HBeAg-positive CHB patients at week 48 and to find a useful predictor for treatment efficacy and investigate individualized plan of antiviral therapy.

PATIENTS AND METHODS

Patients

Ninety-six HBeAg positive CHB patients who have accepted antiviral therapy from September 2010 to October 2011 in Liver Diseases Department of Provincial Hospital affiliated to Shandong University were enrolled in this study. Eighty-six cases were male and 10 cases were female, aged from 20 to 35 years, mean age 27.2 ± 3.2 years. The baseline of ALT was (196 ± 73.6) U/L, HBV DNA were (6.41 ± 0.60) $\log_{10}$ copies/ml, and HBeAg were (569.8 ± 418.1) S/CO. They were categorized into different groups according to the changes of HBeAg at week 24. The groups have no differences in HBeAg, HBV DNA, ALT and sex ratio at baseline. The diagnosis standard is consistent with Chinese Guideline of CHB.² The presence of HBsAg in serum existed for more than 6 months, HBeAg was positive, HBV DNA $>10^4$ copies/ml, the baseline of ALT was between $2 \times$ upper limit

of normal (ULN) and $10 \times$ ULN, TBil $< 2 \times$ ULN, they have not accepted any antiviral therapy or any immunity adjustment therapy one-year before therapy. The contraindication of the antiviral therapy had been excluded.

Methods

Patients were treated with Peg-IFN- α 2a at a dose of $135 \mu\text{g/week}$ (weight ≤ 65 kg) or $180 \mu\text{g/week}$ (weight > 65 kg) for 24 weeks, they were categorized into 3 groups according to the change of HBeAg in week 24: a HBeAg decline $> 2 \log_{10}$ group (group A), a HBeAg decline between $1 \log_{10} - 2 \log_{10}$ (group B), a HBeAg decline $< 1 \log_{10}$ group (group C), group C was randomly distributed into C1 and C2. The patients in group A, group B, and group C1 continued the original therapy and the patients in group C2 were given lamivudine plus Peg-IFN- α 2a for 24 weeks. Serum ALT, HBV DNA, HBsAg, HBeAg and anti-HBe levels were measured at baseline and during therapy (week 12, 24, 36 and 48). ALT was detected by completely automatic biochemistry analyzer. Serum HBsAg, HBeAg and anti-HBe levels were measured by Abbott Architect, results were calculated based on the ratio of the sample (S) to the cut-off (CO) values for each sample and control. Samples

Figure 1. Flowchart of Peg-IFN- α 2a therapy.

Notes: Ninety-six HBeAg positive CHB patients who have accepted Peg-IFN therapy were enrolled, they were categorized into 3 groups according to the change of HBeAg at week 24, a HBeAg decline $> 2 \log_{10}$ group (group A), a HBeAg decline between $1 \log_{10} - 2 \log_{10}$ (group B), a HBeAg decline $< 1 \log$ group (group C), group C was randomly distributed into C1 and C2. The patients in group A, group B, and group C1 continued the original therapy and the patients in group C2 were given lamivudine plus Peg-IFN- α 2a for 24 weeks.

with S/CO <1.0 were considered to be negative for HBeAg. Samples with S/CO <1.0 were considered to be positive for anti-HBe. HBeAg seroconversion was defined as the loss of HBeAg accompanied by detection of anti-HBe. HBV DNA and HBV cccDNA were detected in liver sample by Roche Light-cycler FQ-PCR. At week 48, the treatment efficacy and hepatitis B virus covalently closed circular DNA (HBV cccDNA) in liver biopsies were analyzed (Figure 1).

Statistical analysis

Data analysis was described using means $\pm$ SD or medians (with interquartile ranges). Comparisons between group A, group B and group C were performed using the Nemenyi method. Comparisons between group C1 and group C2 were made using the Mann-Whitney test. Comparisons of HBV DNA loss and HBeAg seroconversion were performed using the Chi-square test. All analyses were performed using SPSS software (Inc., Chicago, IL, USA) for Windows (release 17.0). $P < 0.05$ was considered statistically significant.

RESULTS

Mean reduction of serum HBV DNA at week 48

Group A: 5.8 log₁₀ copies/ml, group B: 3.8 log₁₀ copies/ml, group C1: 2.8 log₁₀ copies/ml, group C2: 5.7 log₁₀ copies/ml. The mean reduction of serum HBV DNA in group A was greater than that in groups B and C1 (Nemenyi display: $\chi^2_{AB}=9.06$, $P < 0.05$; $\chi^2_{AC1}=23.45$, $P < 0.05$), and that in group C1 was greater than that in group C2 (Mann-Whitney U display: $U=44.0$, $W=320.0$, $P_{C1C2}<0.01$), the difference between groups B and C1 had no statistical significance (Nemenyi display: $\chi^2_{BC1}=3.28$, $P = 0.19$). Mean reduction of HBeAg: group A: 2.7 log₁₀ S/CO, group B: 1.9 log₁₀ S/CO, group C1: 0.9 log₁₀ S/CO, group C2: 1.6 log₁₀ S/CO. The differences among groups A, B and C1 (Nemenyi display: $\chi^2_{AB}=10.58$, $P < 0.05$; $\chi^2_{AC1}=43.32$, $P < 0.05$; $\chi^2_{BC1}=10.82$, $P < 0.05$) and between groups C1 and C2 (Mann-Whitney U display: $U=109.0$, $W=385.0$, $P_{C1C2}<0.01$) were statistically significant.

Serum HBeAg kinetics according to the treatment response

Group A had a significant decrease in the first 24 weeks, groups C1 and C2 had a slower decrease in

Figure 2. Kinetics of serum HBeAg.

Notes: Group A had a significant decrease in the first 24 weeks, groups C1 and C2 had a slower decrease in the first 24 weeks in comparison with groups A and B. Group C2 had a more markedly decrease than group C1 after adding lamivudine in the last 24 weeks.

Figure 3. Kinetics of serum HBV DNA.

Notes: Serum HBV DNA kinetics according to the treatment response: group A had a significant decrease in the first 24 weeks, group B had a slower decrease in the first 24 weeks than group A and reached the lower limit of detection of serum HBV DNA in week 36. Groups C1 and C2 had a small decrease in the first 24 weeks, group C2 had a more remarkable decrease than group C1 after adding lamivudine in the last 24 weeks.

the first 24 weeks in comparison with groups A and B. Group C2 had a more markedly decrease than group C1 after adding lamivudine in the last 24 weeks (Figure 2). Serum HBV DNA kinetics according to the treatment response: group A had a significant decrease in the first 24 weeks, group B had a slower decrease in the first 24 weeks than group A and reached the lower limit of detection of serum HBV DNA in week 36. Groups C1 and C2 had a small decrease in the first 24 weeks, Group C2 had a more remarkable decrease than group C1 after adding lamivudine in the last 24

Table 1. The relationship between therapy response at week 48 and serum HBeAg at week 24

Groups	number	HBV DNA undetectable	HBeAg seroconversion	ALT normalization
A	24	87.5% (21/24)	75.0% (18/24)	70.8% (17/24)
B	29	34.5% (10/29)	24.1% (7/29)	69.0% (20/29)
C1	23	17.4% (4/23)	13.0% (3/23)	21.7% (5/23)
χ^2_{AB}	-	15.203	13.632	0.006
P_{AB}	-	<0.01	<0.01	0.939
χ^2_{AC1}	-	23.186	18.240	10.326
P_{AC1}	-	<0.01	<0.01	<0.01
χ^2_{BC1}	-	1.904	1.016	11.460
P_{BC1}	-	0.168	0.313	<0.01

Table 2. The effect of adding lamivudine in group C2

Groups	number	HBV DNA undetectable	HBeAg seroconversion	ALT normalization
C1	23	17.4% (4/23)	13.0% (3/23)	21.7% (5/23)
C2	20	85.0% (17/20)	25.0% (5/20)	40.0% (8/20)
χ^2	-	19.570	1.010	1.691
P	-	$P < 0.01$	0.315	0.193

Table 3. The quantity of HBV cccDNA in liver tissue at week 48 in four groups [M(QR)]

Groups	number	week	HBV cccDNA at week 48 (copies/mg)
A	8	48	30.5 (232.0)
B	9	48	412.0 (278.0)
C1	7	48	1260.0 (4572.0)
C2	7	48	1380.0 (3637.0)

weeks (Figure 3).

Therapy effects in each group

At week 48, HBV DNA undetectable rates in group A, group B, group C1 and group C2 were 87.5% (21/24), 34.5% (10/29), 17.4% (4/23) and 85.0% (17/20) respectively. HBV DNA undetectable rate in group A was greater than in groups B and C1 ($\chi^2_{AB}=15.203$, $P < 0.01$; $\chi^2_{AC1}=23.186$, $P < 0.01$), that in group C1 was greater than in group C2 ($\chi^2=19.570$, $P < 0.01$), the difference between groups B and C1 had no statistical significance ($\chi^2_{BC1}=1.904$, $P = 0.168$). HBeAg seroconversion rates were 75.0% (18/24), 24.1% (7/29), 13.0% (3/23) and 25.0% (5/20) respectively, that in group A was greater than in groups B and C1 ($\chi^2_{AB}=13.632$, $P < 0.01$; $\chi^2_{AC1}=18.240$, $P < 0.01$). The difference between groups B and C1 and between groups C1 and C2 had no statistical significance ($\chi^2_{BC1} = 1.016$, $P = 0.313$; $\chi^2_{C1C2} = 1.010$, $P = 0.315$). ALT normalization rate in group A, group B, group C1 and group C2 were 70.8% (17/24), 69.0% (20/29), 21.7% (5/23) and 40.0% (8/20) respectively, and

Table 4. Untoward effect during the therapy

Groups	flu like symptoms	neutropenia	thrombo- cytopenia
A	91.7% (22/24)	50.0% (12/24)	8.3% (2/24)
B	86.2% (25/29)	48.3% (14/29)	10.3% (3/29)
C1	78.3% (18/23)	56.5% (13/23)	8.7% (2/23)
C2	95.0% (19/20)	45.0% (9/20)	20.0% (4/20)
χ^2	3.249	0.626	1.878
P	0.355	0.891	0.598

that in group A was greater than in groups B and C1 ($\chi^2_{AC1} = 10.326$, $P < 0.01$; $\chi^2_{BC1} = 11.460$, $P = 0.168$). The difference between groups B and C1 and between groups C1 and C2 had no statistical significance ($\chi^2_{AB} = 0.006$, $P = 0.939$; $\chi^2_{C1C2} = 1.691$, $P = 0.193$) (Table 1 and Table 2).

Therapy effects on cccDNA

At week 48, group A had lower HBV cccDNA in liver tissue than group B and group C1 (Nemenyi display: $\chi^2_{AB} = 6.78$, $P < 0.05$; $\chi^2_{AC1} = 11.63$, $P < 0.05$). The difference of HBV cccDNA between groups B and C1 or between groups C1 and C2 had no statistical significance (Nemenyi display: $\chi^2_{BC1} = 0.60$, $P = 0.74$; Mann-Whitney U display: $U=109.0$, $W=385.0$, $P_{C1C2} < 0.01$) (Table 3).

Safety

The frequency and severity of adverse events were

usually transient. In 1-4 weeks, the most frequent serious adverse events occurred in eighty-four patients were fever, lacking in strength and flu like symptoms, the adverse events reduced during the prolonged treatment time. At week 48, 50% of patients had neutropenia, 11.5% of patients had thrombocytopenia. Serious myelosuppression, liver function damage, thyrotoxicosis and psychosis were not observed during the treatment. The groups did not differ in the incidence of fever, lacking in strength, flu like symptoms, neutropenia or thrombocytopenia (Table 4).

DISCUSSION

Reduction of HBV DNA is an excellent indicator of antiviral efficacy. However, a decrease in HBV DNA alone may be transient, particularly once antiviral agents are discontinued.^{3,4} Seroconversion of HBeAg is another surrogate endpoint that is considered to be a marker for durable therapeutic response and had decreased liver-related morbidity and mortality.^{5,6} The evaluation standards of antiviral therapy are the decrease of HBV DNA and HBeAg, HBV DNA undetectable rate, HBeAg seroconversion rate, ALT normalization rate and HBV cccDNA.⁷

To assess on-treatment serum HBeAg kinetics to predict outcomes of interferon antiviral therapy has been found useful for the realization of individual antiviral treatment. Previous studies have identified that HBeAg at baseline can be a predictor for the treatment effect of Peg-IFN- α 2a on HBeAg-positive CHB patients⁸. In this study, CHB patients who showed an HBeAg decline of $> 2 \log_{10}$ had a higher HBeAg and HBV DNA decrease, a higher HBV DNA undetectable rate and HBeAg seroconversion rate, and a lower HBV cccDNA than those who showed an HBeAg decline of $< 2 \log_{10}$ at week 24. CHB patients who showed an HBeAg decline of $> 2 \log_{10}$ at week 24 of Peg-IFN- α 2a treatment had better treatment outcomes at week 48 than those who showed an HBeAg decline of $< 2 \log_{10}$ at week 24.

To investigate individualized plan of antiviral therapy, the patients in group C2 were given lamivudine plus Peg-IFN- α 2a for 24 weeks, the results showed that the combination of Peg-IFN- α 2a and lamivudine can improve the clinical responses,

group C2 had a higher HBeAg and HBV DNA decrease and a higher HBV DNA undetectable rate than group C1. Previous studies have proved several factors that were associated with a higher likelihood of HBeAg seroconversion, including low serum HBV DNA levels after antiviral therapy and prolonged antiviral therapy. So it is necessary to prolong the combined antiviral therapy and investigate the value of it.⁹

In conclusion, CHB patients who showed an HBeAg decline of $> 2 \log_{10}$ at week 24 with Peg-IFN- α 2a treatment had better treatment outcome at week 48 than those who showed HBeAg decline $< 2 \log_{10}$ at week 24. Augmenting the Peg-IFN- α 2a treatment with lamivudine could improve the clinical response. A change of quantifiable HBeAg at week 24 of Peg-IFN- α 2a treatment may be a useful predictor of therapeutic efficacy of 48-week antiviral regimen.

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