ORIGINAL ARTICLES—LIVER, PANCREAS AND BILIARY TRACT

Early Hepatitis B Virus DNA Suppression Can Predict Virologic Response to Peginterferon and Lamivudine Treatment

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Background & Aims: **We aimed to investigate the early on-treatment HBV DNA response to predict sustained virologic response for peginterferon and lamivudine combination therapy.** ***Methods:*** **Patients recruited in previous clinical trials receiving 32-week peginterferon alfa-2b and 52- to 104-week lamivudine combination treatment were studied. The areas under the receiver operating characteristic curve (ROC) of HBV DNA at different time intervals were analyzed to predict sustained virologic response, which was defined as HBeAg seroconversion and HBV DNA <10,000 copies/mL at 1 year after treatment.** ***Results:*** **Fifty-seven patients had peginterferon started 8 weeks before lamivudine, and 9 patients had the 2 drugs commenced simultaneously. Eighteen (27%) patients developed sustained virologic response. The area under ROC for log HBV DNA to predict sustained virologic response reached 0.80 (95% confidence interval, 0.69–0.91; $P < .001$) at week 8 and plateaued off at subsequent time intervals. Among the 57 patients started with peginterferon monotherapy during the first 8 weeks, the area under ROC was 0.83 (95% confidence interval, 0.73–0.94; $P < .001$). Compared with other time intervals, the likelihood ratio for sustained virologic response was highest for HBV DNA <10,000 copies/mL at week 8 (10.35), with a high sensitivity (0.89) and negative predictive value (0.92). Two of 33 (6%) patients who had HBV DNA ≥10,000 copies/mL at week 8 developed sustained virologic response.** ***Conclusions:*** **HBV DNA ≥10,000 copies/mL at week 8 of peginterferon treatment had a low chance of sustained virologic response.**

Chronic HBV infection is the most important cause of liver cirrhosis and hepatocellular carcinoma in most Asian countries.[1] Positive HBeAg and high HBV DNA are risk factors for liver cirrhosis and hepatocellular carcinoma.[2–4] Spontaneous HBeAg seroconversion is associated with reduced risk of liver-related complications.[5,6] Although the rate of HBeAg seroconversion is low with interferon-alfa treatment, sustained responders have a significantly reduced risk of liver-related complication as compared with the nonresponders or untreated patients.[7–9] So far, there is only one phase 2 study suggesting that peginterferon-alfa has a higher sustained virologic response than the conventional interferon-alfa.[10] Nonetheless, peginterferon-alfa has become the preferred treatment option as a result of its more convenient once weekly dosing. In contrast to the high rate of post-treatment relapse after nucleoside analogues, response to peginterferon treatment tends to be more sustained.[11]

One major problem of peginterferon treatment is its relatively low sustained response rate. The rate of sustained HBeAg seroconversion after 32-week to 48-week peginterferon-alfa treatment, either alone or in combination with lamivudine, is approximately 33%.[12–14] Combination of lamivudine with peginterferon only improves the on-treatment HBV DNA suppression but does not improve the sustained off-treatment virologic response.[13,14] High pretreatment serum ALT level and low HBV DNA were found associated with higher 6-month post-treatment sustained response in 2 large multicenter studies, but it cannot be confirmed by our study with longer post-treatment follow-up.[11,13,14] In a recent study comparing 3 different combination regimes of peginterferon and lamivudine for 2 years, we found that low serum HBsAg level, which might reflect a lower level of the covalently closed circular DNA level inside the hepatocytes, was associated with higher sustained virologic response.[15] The prediction of these pretreatment clinical factors is too rough and cannot be used to select or preclude patients from receiving peginterferon treatment.

Limited by the numerous adverse effects, inconvenient subcutaneous route of administration, and the high cost of peginterferon treatment, it will be desirable if nonresponders can be identified early, so that the treatment can be stopped to avoid unnecessary dosing and suffering. In chronic hepatitis C, failure to achieve early virologic response at week 12 is used as the early treatment stopping rule.[16] In this study, we investigated the on-treatment HBV DNA response to peginterferon and lamivudine combination treatment and their ability to predict sustained virologic response. We aimed to determine a certain HBV DNA level at an early stage of treatment that could predict future nonresponders.

Abbreviations used in this paper: Anti-HBe, antibody to hepatitis B e antigen; CI, confidence interval; ROC, receiver operating characteristic curve.

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Patients and Methods

Patients

Chinese chronic hepatitis B patients who received peginterferon alfa-2b (PegIntron; Schering-Plough Corporation, Kenilworth, NJ) and lamivudine (Zeffix; GlaxoSmithKline, Middlesex, UK) combination treatment in Prince of Wales Hospital, Hong Kong, were studied. They were HBeAg-positive patients who had completed per protocol treatment in 2 previously conducted clinical trials.[12,17] All patients had peginterferon started 8 weeks before the commencement of lamivudine or simultaneously with lamivudine. Peginterferon was prescribed for 32 weeks and stopped. Lamivudine was continued for 52 weeks in one study and 104 weeks in the other study. Liver biopsy was performed at baseline and at the end of treatment. Histologic necroinflammation (0–18) and liver fibrosis (0–6) were scored by Ishak scoring system (0–6). All patients were followed up regularly at 3-month intervals after cessation of lamivudine treatment.

Serum biochemistry, HBeAg, hepatitis B e antibodies (anti-HBe), and HBV DNA were regularly monitored during and after treatment. HBV genotype and quantitative HBsAg level were determined at the baseline samples. Sustained virologic response was defined as HBeAg seroconversion (with appearance of anti-HBe) and HBV DNA lower than 10,000 copies/mL at 1 year after stopping of treatment.[18]

Laboratory Assays

Hepatitis B surface antigen assay. Residual serum samples were stored in −80°C freezer. All baseline samples at the time of liver biopsy were tested and quantified for HBsAg by Architect HBsAg QT (Abbott Diagnostics, Wiesbaden, Germany) according to the manufacturer's instructions.[19] The sensitivity of the Architect assay ranged from 0.05–250 IU/mL. Samples with HBsAg titer higher than 250 IU/mL were diluted to 1:500–1000 to bring the reading to the range of the calibration curve.

Hepatitis B virus DNA assay and genotyping. HBV DNA was extracted by QIAGEN QIAamp DNA Mini Kit (QIAGEN Inc, Chatsworth, CA) according to the instructions of the manufacturer. HBV DNA was quantified by TaqMan real-time polymerase chain reaction assay as described previously.[20] The range of HBV DNA detection was from 10^2–10^9 copies/mL. HBV genotyping was determined by restriction fragment length polymorphism and confirmed by direct sequencing in case of doubt in the residual serum sample at the initial visit as described previously.[21,22]

Statistical Analysis

Statistical tests were performed by SPSS (version 11.0; Chicago, IL). Continuous variables were expressed as mean ± standard deviation or median (range) as appropriate. HBV DNA and HBsAg were logarithmically transformed for analysis. Continuous variables were compared by Mann-Whitney U test. Categorical variables were compared by Pearson χ^2 test or Fisher exact test as appropriate. Area under the receiver operator characteristic curve (ROC) was performed to assess the predictive values of HBV DNA and HBsAg for sustained virologic response. Sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio were evaluated for various HBV DNA levels at different time intervals for sustained virologic response. Statistical significance was taken as a P value less than .05. All statistical tests were two-sided.

Results

Baseline Characteristics

On the basis of the 2 previous clinical trials, 66 patients were eligible for this analysis. Forty-eight (73%) patients received 32 weeks of peginterferon and 52 weeks of lamivudine. Eighteen (27%) patients received 32 weeks of peginterferon and 104 weeks of lamivudine (9 patients had peginterferon started 8 weeks before lamivudine, and 9 patients had the 2 drugs started simultaneously). Forty-eight (73%) patients were infected by genotype C HBV, whereas the remaining 18 (27%) patients were infected by genotype B HBV. Eighteen (27%) patients developed sustained virologic response (Table 1). Baseline clinical factors including patient demographics, HBV genotypes, transaminase levels, liver histology, and treatment regimes were not associated with sustained virologic response (Table 1). There was a trend for lower HBV DNA and lower HBsAg levels at pretreatment to predict sustained virologic response, but the associations fell just short of statistical significance. The area under ROC to predict sustained virologic response for log HBV DNA was 0.65 (95% confidence interval [CI], 0.50–0.80; $P = .067$), and that for log HBsAg was 0.65 (95% CI, 0.50–0.79; $P = .070$).

Table 1. Clinical Characteristics of Sustained Responders and Nonsustained Responders at Baseline

	Overall	Sustained responder	Nonsustained responder	*P* value
No. of patients	66	18	48	
Age (*y*)	32 ± 9	31 ± 9	32 ± 9	.74
Male (*n*, %)	39 (59%)	10 (56%)	29 (60%)	.72
Body mass index (kg/m^2)	22.8 ± 3.7	23.6 ± 4.4	22.4 ± 3.4	.16
Log HBV DNA (*copies/mL*)	8.28 ± 0.98	7.99 ± 0.89	8.39 ± 1.00	.067
Log HBsAg (*IU/mL*)	3.83 ± 0.74	3.57 ± 0.68	3.92 ± 0.75	.070
ALT (*IU/L*)	144 (48–2260)	140 (57–420)	152 (48–2260)	.67
HBV genotype C (*n*, %)	48 (73%)	13 (72%)	35 (73%)	.96
Necroinflammatory score	5 (1–15)	6 (1–11)	5 (1–15)	.66
Fibrosis score	1 (0–6)	1 (0–4)	1 (0–6)	.68
Staggered regime (*n*, %)	57 (87%)	14 (78%)	43 (90%)	.21
2-year treatment (*n*, %)	18 (27%)	5 (28%)	13 (27%)	.96