Background: Although pegylated interferon (PEG-IFN) and nucleotide/nucleoside analogue (NA) combination therapy is considered to be optimal for accelerating serum hepatitis B surface antigen (HBsAg) reduction, the effect is limited, and the best approach to PEG-IFN treatment for chronic hepatitis B patients during long-term NA therapy has yet to be determined.

Methods: A total of 21 hepatitis B e antigen-negative chronic hepatitis B patients whose HBV DNA levels were suppressed to undetectable levels by NA therapy were administrated PEG-IFN-α2a for 48 weeks (sequential therapy: 10, add-on therapy: 11). Factors associated with HBsAg reduction by PEG-IFN therapy were analysed.

Results: During PEG-IFN treatment, HBsAg levels were reduced by 0.48 log IU/ml. More than 1 log IU/ml HBsAg reduction was observed in eight patients (sequential therapy: six, add-on therapy: two), and one patient with sequential therapy achieved HBsAg loss. By univariate analysis, sequential therapy was marginally associated with more than 1 log IU/ml HBsAg reduction during PEG-IFN treatment ($P=0.060$). After PEG-IFN treatment, only five patients, including the patient with HBsAg loss, achieved more than 0.5 log IU/ml of HBsAg reduction by 1 year after PEG-IFN treatment. By univariate analysis, sequential therapy was significantly associated with HBsAg reduction after PEG-IFN treatment ($P=0.012$). In addition, alanine aminotransferase elevation during PEG-IFN therapy and lower serum interleukin-8 level at the end of PEG-IFN treatment were also significantly associated with HBsAg reduction by 1 year after PEG-IFN treatment ($P=0.038$, $P=0.044$, respectively).

Conclusions: Sequential therapy may be superior to add-on therapy in reducing HBsAg levels during long-term NA therapy in chronic hepatitis B patients.

Introduction

HBV infection is a serious global health problem. More than 2 billion people have been infected with HBV, and about 20% remain chronically infected [1,2]. Chronically infected individuals often develop chronic hepatitis, liver cirrhosis and hepatocellular carcinoma, and the incidence of hepatocellular carcinoma in chronically infected individuals is significantly higher than that in healthy individuals [3]. Once HBV infects human hepatocytes, HBV genomes are transported into the nucleus, and some viral genomes become integrated into human chromosomes [4–7]. Thus, complete elimination of the virus is difficult, and patients are generally treated with interferon (IFN) and nucleotide/nucleoside analogues (NAs), which suppress viral replication and
prevent the progression of liver disease by combating inflammation [8–10].

Japanese guidelines from the Japan Society of Hepatology currently recommend that the final goal of antiviral therapy for chronic hepatitis B should be HBsAg loss [9]. However, it is difficult to achieve HBsAg loss by long-term treatment with NAs, and the number of patients who do achieve HBsAg loss is limited under the present antiviral treatments. Recently, Marcellin et al. [11] performed an open-label, randomized, controlled study in which chronic hepatitis B patients were treated with tenofovir disoproxil fumarate (TDF) and/or pegylated IFN (PEG-IFN)-α2a, and they reported that TDF and PEG-IFN-α2a combination therapy was more efficient for inducing HBsAg loss than TDF or PEG-IFN-α2a monotherapy. While no patient achieved HBsAg loss by TDF monotherapy alone in this study, the results indicated that PEG-IFN-α2a might be a key drug for inducing HBsAg loss or reduction in chronic hepatitis B patients.

Results of the study by Marcellin et al. [11] suggest that PEG-IFN-α2a therapy can induce HBsAg loss or HBsAg reduction in chronic hepatitis B patients who have been unable to achieve HBsAg loss in spite of long-term NA therapy. To analyse the effects on HBsAg levels by PEG-IFN-α2a therapy, we treated chronic hepatitis B patients who have been treated with NAs for more than 1 year with PEG-IFN-α2a and analysed HBsAg levels during and after PEG-IFN treatment.

Methods

Patients

A total of 21 Japanese chronic hepatitis B patients were enrolled. All patients had been treated with NAs for more than 12 months at Hiroshima University Hospital or Kawakami Clinic in Hiroshima, Japan. None of the patients were infected with other viruses, including HIV or HCV, or had evidence of other liver diseases, such as autoimmune hepatitis or alcoholic liver disease. Patients with total ethanol intake of more than 100 kg were excluded [12]. All patients gave written informed consent to participate in the study. The experimental protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the ethical committee of Hiroshima University Hospital (Approval ID: E-463).

After obtaining informed consent, ten patients were treated using sequential therapy, which included 48 weeks of PEG-IFN-α2a therapy (180 µg/week) beginning 1 month prior to discontinuation of NA therapy until 11 months after. Although the optimal duration of overlap of PEG-IFN and NA therapy was not established, we determined the overlap duration following previous reports in which sequential studies were performed using conventional IFNs [13–15]. The remaining 11 patients were treated using add-on therapy, which included 48 weeks of PEG-IFN-α2a that overlapped with NA combination therapy.

Blood samples were obtained from the patients at the beginning of PEG-IFN therapy and every 4 weeks during the follow-up period. Biochemical and haematological tests were performed in the Hiroshima University Hospital laboratory. Remaining serum was stored at -80°C for further analysis.

Measurement of serum HBV markers

HBV DNA levels were quantified by real-time PCR using the TaqMan PCR System (Roche Diagnostics, Tokyo, Japan). Hepatitis B e antigen (HBeAg) and hepatitis B surface antigen (HBsAg) levels were measured by Chemiluminescent Immuno Assay (CLIA) using the ARCHITECT analyzer (Abbott Japan Co., Ltd, Tokyo, Japan).

Measurement of serum IL-8 levels

Serum interleukin (IL)-8 levels were measured by AlphaLISA immunoassay kit according to the manufacturer’s instructions. The quantitative range for serum IL-8 was 0.001–100,000 pg/ml.

Statistical analysis

The baseline characteristics of the patients in the two groups were compared, and differences were assessed by χ² test with Yate’s correction, the Fisher’s exact probability test, or the Mann–Whitney U test, as appropriate. All P-values less than 0.05 by two-tailed test were considered significant. Variables with at least marginal significance (P<0.10) in univariate analysis were entered into multiple logistic regression analysis to identify independent factors. Statistical analysis was performed using SPSS® ver. 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of study patients

The clinical background of the 21 enrolled patients at the beginning of PEG-IFN therapy is shown in Additional file 1. Two patients were treated with 100 mg/day of lamivudine (LMV), 13 were treated with 0.5 mg/day of entecavir (ETV), 5 were treated with 100 mg/day of LMV plus 10 mg/day of adefovir (ADV) combination therapy, and one was treated with 100 mg/day of LMV plus 300 mg/day of TDF combination therapy. 17 patients were male and 4 were female. All 21 patients were negative for HBeAg. During PEG-IFN treatment, no patient needed PEG-IFN dose reduction due to side effects. There was no significant difference among clinical factors, such as alanine aminotransferase (ALT), HBsAg, hepatitis B core related antigen (HBcAg) at the beginning of PEG-IFN therapy between patients treated
with sequential therapy and those treated with add-on therapy. After PEG-IFN therapy, NA treatment was continued in the 11 patients with add-on therapy, whereas the 10 patients treated with sequential therapy were followed-up without antiviral treatment after PEG-IFN therapy. Three patients were retreated with NAs within 2 years after the sequential therapy, but the remaining seven patients were followed-up without antiviral therapy more than 2 year after sequential therapy.

Analysis of HBsAg reduction during PEG-IFN therapy

The changes in HBsAg levels during PEG-IFN therapy are shown in Additional file 2. During PEG-IFN treatment, HBsAg levels were reduced by 0.48 log IU/ml. More than 1 log IU/ml of HBsAg reduction was observed in 8 of 21 patients, and the reduction in HBsAg levels in the remaining 13 patients was less than 1 log IU/ml. To identify factors associated with >1 log IU/ml of HBsAg reduction during PEG-IFN therapy, the patients were divided into two groups based on HBsAg reduction levels during PEG-IFN therapy (>1 log IU/ml and ≤1 log IU/ml). As shown in Table 1, the proportion of patients who achieved >1 log IU/ml of HBsAg reduction following sequential therapy was marginally higher than that of patients treated with add-on therapy (P=0.063), and sequential therapy was also observed as a marginally associated factor for HBsAg reduction during PEG-IFN therapy by multivariate analysis (P=0.060). However, the duration of NA treatment, HBsAg, HBcAg and HBV DNA levels at the beginning of PEG-IFN treatment were not significant.

Analysis of HBsAg reduction after PEG-IFN therapy

Time courses of HBsAg levels during and after PEG-IFN therapy are shown in Figure 1. Since the antiviral effects of IFN are considered to continue after the end of IFN treatment, we compared the changes of HBsAg levels for 1 year after PEG-IFN therapy. Although greater than 0.5 log IU/ml of HBsAg reduction after PEG-IFN therapy was observed in 5 out of 10 patients who were treated with sequential therapy (Figure 1A), HBsAg reduction after PEG-IFN therapy was not observed in patients with add-on therapy (Figure 1B). To identify factors associated with more >0.5 log IU/ml of HBsAg reduction 1 year after PEG-IFN therapy, we performed univariate analysis using clinical factors. As shown in Table 2, >0.5 log HBsAg reduction at 1 year after PEG-IFN therapy was significantly associated with sequential therapy (P=0.012) and was marginally associated with ALT and HBsAg levels at the beginning of PEG-IFN therapy (P=0.050, P=0.062, respectively). To identify independent predictive factors for HBsAg reduction after PEG-IFN therapy, multivariate analysis using multiple logistic regression analysis was attempted but was unsuccessful due to the small number of study subjects.

Within 1 year after PEG-IFN therapy, no elevation of serum ALT and HBV DNA levels occurred in any of the 11 patients who were treated with add-on therapy. However, serum ALT elevation occurred in 4 of 10 patients who were treated using sequential therapy, and HBV DNA elevation (>3.0 log copies/ml) occurred in 4 of 10 patients with sequential therapy.

**Table 1. Statistical analysis of factors associated with >1 log IU/ml reduction of HBsAg during PEG-IFN treatment (n=21)**

<table>
<thead>
<tr>
<th>Factors</th>
<th>HBsAg reduction ≥1 log (n=8)</th>
<th>HBsAg reduction &lt;1 log (n=13)</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male:female</td>
<td>8.0</td>
<td>9.4</td>
<td>0.119</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>51.5 (41–73)</td>
<td>55.0 (41–70)</td>
<td>0.645*</td>
<td></td>
</tr>
<tr>
<td>Before IFN treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets, ×10⁹/µl</td>
<td>20.4 (16.1–25.4)</td>
<td>16.4 (13.0–35.4)</td>
<td>0.268*</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin, mg/dl</td>
<td>0.6 (0.4–2.0)</td>
<td>0.6 (0.4–1.1)</td>
<td>0.750*</td>
<td></td>
</tr>
<tr>
<td>ALT, IU/l</td>
<td>20 (13–61)</td>
<td>16 (12–37)</td>
<td>0.645*</td>
<td></td>
</tr>
<tr>
<td>Alb, g/dl</td>
<td>4.6 (3.5–6.0)</td>
<td>4.4 (4.1–5.8)</td>
<td>0.301*</td>
<td></td>
</tr>
<tr>
<td>HBsAg, log IU/ml</td>
<td>2.41 (0.3–4.2)</td>
<td>2.96 (0.9–3.8)</td>
<td>0.268*</td>
<td></td>
</tr>
<tr>
<td>HBcAg, log IU/ml</td>
<td>&lt;3.0 (&lt;3.0–3.6)</td>
<td>&lt;3.0 (&lt;3.0–4.0)</td>
<td>0.210*</td>
<td></td>
</tr>
<tr>
<td>HBV DNA, log copies/ml</td>
<td>undet (undet–&lt;2.1)</td>
<td>undet (undet–&lt;2.1)</td>
<td>0.654*</td>
<td></td>
</tr>
<tr>
<td>HBV genotype, B:C</td>
<td>1:7</td>
<td>2:11</td>
<td>0.684</td>
<td></td>
</tr>
<tr>
<td>NA treatment, LMV/LMV+ADV:ETV:LMV+TDF</td>
<td>2.2:4:0</td>
<td>0.3:9:1</td>
<td>0.335</td>
<td></td>
</tr>
<tr>
<td>Duration of NA treatment, months</td>
<td>87.9 (34.7–137.4)</td>
<td>77.8 (23.5–156.8)</td>
<td>0.374*</td>
<td></td>
</tr>
<tr>
<td>Duration of IFN treatment, weeks</td>
<td>48 (48–48.4)</td>
<td>48 (48–49.4)</td>
<td>0.750*</td>
<td></td>
</tr>
<tr>
<td>IFN treatment, sequential:add-on</td>
<td>6.2</td>
<td>4.9</td>
<td>0.063</td>
<td>0.060</td>
</tr>
</tbody>
</table>

Data are median (range) unless otherwise indicated. Univariate analysis was performed by χ² test with Yate’s correction, the Fisher’s exact probability test or the Mann-Whitney U test, as appropriate. Multivariate analysis was performed by multiple logistic regression analysis. ADV, adefovir; Alb, albumin; AL, alanine aminotransferase; ETV, entecavir; HBcAg, hepatitis B core related antigen; HBsAg, hepatitis B surface antigen; LMV, lamivudine; NA, nucleotide/nucleoside analogue; PEG-IFN, pegylated interferon; TDF, tenofovir disoproxil fumarate; undet, undetermined.
Figure 1. Change in HBsAg level in study patients during and after PEG-IFN treatment

Hepatitis B surface antigen (HBsAg) levels of patients who were treated with (A) sequential therapy and with (B) add-on therapy are shown. The bold lines indicate patients whose HBsAg levels became negative or were reduced more than 0.5 log IU/ml after pegylated interferon (PEG-IFN) treatment. NAs, nucleotide/nucleoside analogues; w, week.

Table 2. Univariate analysis of factors associated with ≥0.5 log IU/ml reduction of HBsAg after PEG-IFN treatment (n=21)

<table>
<thead>
<tr>
<th>Factors</th>
<th>HBsAg reduction ≥0.5 log (n=5)</th>
<th>HBsAg reduction &lt;0.5 log (n=16)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male:female</td>
<td>5:0</td>
<td>12:4</td>
<td>0.304</td>
</tr>
<tr>
<td>Age, years</td>
<td>51 (41–56)</td>
<td>54 (41–73)</td>
<td>0.208*</td>
</tr>
<tr>
<td>Before IFN treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets, ×10^4/µl</td>
<td>20.4 (16.1–25.4)</td>
<td>16.4 (13.0–35.4)</td>
<td>0.398</td>
</tr>
<tr>
<td>Total bilirubin, mg/dl</td>
<td>0.6 (0.6–2.0)</td>
<td>0.5 (0.4–1.1)</td>
<td>0.208*</td>
</tr>
<tr>
<td>ALT, IU/l</td>
<td>24 (16–61)</td>
<td>16 (12–37)</td>
<td>0.050*</td>
</tr>
<tr>
<td>Alb, g/dl</td>
<td>4.4 (4.3–4.8)</td>
<td>4.5 (3.9–5.0)</td>
<td>0.603*</td>
</tr>
<tr>
<td>HBsAg, log IU/ml</td>
<td>2.25 (0.3–3.4)</td>
<td>2.96 (2.1–4.2)</td>
<td>0.062*</td>
</tr>
<tr>
<td>HBCrAg, log U/ml</td>
<td>&lt;3.0 (&lt;3.0–3.6)</td>
<td>&lt;3.0 (&lt;3.0–4.0)</td>
<td>0.460*</td>
</tr>
<tr>
<td>HBV DNA, log copies/ml</td>
<td>undet (undet&lt;2.1)</td>
<td>undet (undet&lt;2.1)</td>
<td>0.823*</td>
</tr>
<tr>
<td>HBV genotype, B:C</td>
<td>0.5</td>
<td>3:13</td>
<td>0.421</td>
</tr>
<tr>
<td>NA treatment, LMV:LMV+ADV:ETV:LMV+TDF</td>
<td>1:2:2:0</td>
<td>1:3:1:1</td>
<td>0.499</td>
</tr>
<tr>
<td>Duration of NA treatment, months</td>
<td>82.0 (49.6–99.7)</td>
<td>89.9 (23.5–156.8)</td>
<td>0.780</td>
</tr>
<tr>
<td>Duration of IFN treatment, weeks</td>
<td>48 (48–48.4)</td>
<td>48 (48–48.4)</td>
<td>0.228*</td>
</tr>
<tr>
<td>IFN treatment, sequential:add-on</td>
<td>5:0</td>
<td>5:11</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Data are median (range) unless otherwise indicated. Univariate analysis was performed by χ^2 test with Yate’s correction, the Fisher’s exact probability test or the Mann-Whitney U test, as appropriate. ADV, adefovir; Alb, albumin; ALT, alanine aminotransferase; ETV, entecavir; HBCrAg, hepatitis B core related antigen; HBsAg, hepatitis B surface antigen; LMV, lamivudine; NA, nucleotide/nucleoside analogue; PEG-IFN, pegylated interferon; TDF, tenofovir disoproxil fumarate; undet, undetermined.
Discovery of novel biomarkers for predicting HBsAg reduction after PEG-IFN therapy

Recently, we found that HBV infection induces the transcriptional activation of IL-8 in human hepatocytes, and we proposed that upregulation of IL-8 might lead to suppression of IFN responsiveness in hepatocytes [16]. Therefore, we predicted that serum IL-8 levels might be associated with the antiviral effect of PEG-IFN, and we measured serum IL-8 levels at the beginning and the end of PEG-IFN therapy. Serum IL-8 levels at the beginning of PEG-IFN therapy did not differ between patients with >0.5 log IU/ml of HBsAg reduction after PEG-IFN therapy and those without (Figure 2A). However, serum IL-8 levels at the end of PEG-IFN therapy in patients with HBsAg reduction were significantly lower than those in patients without HBsAg reduction ($P=0.044$; Figure 2B).

Observing the time course of ALT during PEG-IFN therapy, we found that HBsAg reduction levels from the beginning of therapy to 1 year after PEG-IFN therapy were significantly correlated with the maximum ALT levels during PEG-IFN therapy ($P=0.002$; Figure 3A). In patients whose ALT levels were elevated by more than 50 U/l during PEG-IFN therapy, HBsAg levels were reduced by -1.60 log IU/ml (-0.27 to -3.47) during or after PEG-IFN therapy. However, the reduction of HBsAg levels in patients without ALT elevation during PEG-IFN therapy was significantly lower (-0.38 log IU/ml [-2.30 to -0.11]; $P=0.038$; Figure 3B).

As serum IL-8 levels at the beginning of PEG-IFN therapy and ALT elevation during PEG-IFN therapy were associated with HBsAg reduction during and after PEG-IFN therapy, we analysed the association between serum IL-8 levels and ALT elevation during PEG-IFN therapy. However, serum IL-8 levels at the beginning and end of PEG-IFN therapy were not associated with ALT elevation during PEG-IFN therapy ($P=0.533$, $P=0.203$, respectively; Additional file 3). To predict ALT elevation during PEG-IFN therapy, we also performed statistical analysis using clinical factors; however, no significant differences were observed among factors. Only sequential therapy was identified as a marginally

![Graph A](image1.png)

![Graph B](image2.png)

Figure 2. The association between serum IL-8 levels and HBsAg reduction levels after PEG-IFN treatment

(A) Before interferon (IFN) treatment. (B) At end of IFN treatment. Serum interleukin (IL)-8 levels were measured and compared between patients whose hepatitis B surface antigen (HBsAg) levels were reduced more than 0.5 log IU/ml at 1 year after pegylated interferon (PEG-IFN) treatment and patients whose HBsAg levels were not reduced. Statistical analysis was performed using the Mann-Whitney U test.
Discussion

Revised guidelines for antiviral therapy for chronic hepatitis B have recently been established, and HBsAg loss was designated as the final goal of antiviral therapy [17,18]. However, despite long-term antiviral treatment with NAs, it is difficult to induce HBsAg loss by NA therapy alone. Marcellin et al. [11] performed a randomized, controlled study involving chronic hepatitis B patients with no history of NA treatment that showed the superiority of TDF and PEG-IFN-α2a combination therapy for inducing HBsAg loss. However, it is unclear whether add-on therapy, in which 48 weeks of PEG-IFN-α2a therapy is administered simultaneously with ongoing long-term NA therapy, is superior to sequential therapy, in which 48 weeks of PEG-IFN-α2a therapy (180 µg/week) are administered from 1 month prior to discontinuation of long-term NA therapy to 11 months post-discontinuation, for HBsAg reduction. In this pilot study, we compared the effects of HBsAg reduction between sequential therapy and add-on therapy in chronic hepatitis B patients who were treated with NAs for more than 1 year, and we compared HBsAg reduction levels between these PEG-IFN therapies.

As shown in Additional file 2, greater than 1 log IU/ml of HBsAg reduction was observed in 38.1% (8/21) of patients during PEG-IFN therapy, but HBsAg levels in the remaining 13 patients were not reduced by PEG-IFN therapy. Antiviral effects of PEG-IFN have been reported to be significantly associated with higher M2BPGi levels, higher ALT levels, lower HBsAg levels, lower HBeAg levels and lower HBV DNA levels at the beginning of PEG-IFN therapy [19]. However, we could not identify a useful predictive clinical factor for HBsAg reduction in this study. In the clinical study by Zhu et al. [19], patients whose HBsAg levels were more than 3 log IU/ml were enrolled. However, in 66.7% of our study patients, HBsAg levels had already declined to less than 3 log IU/ml during long-term NA therapy.
Antiviral effects can be observed for a long time after the cessation of IFN administration [20–23]. To investigate the subsequent antiviral effects of PEG-IFN therapy, we analysed HBsAg reduction levels from the end of PEG-IFN therapy to 1 year after the therapy. As shown in Figure 1, HBsAg levels in 16 of 21 patients were steady or gradually elevated after the cessation of PEG-IFN treatment, and HBsAg levels in only 5 remaining patients were reduced after PEG-IFN treatment. To identify predictive factors for HBsAg reduction after the cessation of PEG-IFN treatment, we performed univariate analysis, and only sequential therapy was found to be significant (Table 2). Although there are several reports in which antiviral effects of PEG-IFN therapy, including sequential or add-on therapy, were analysed [11,14,24], the superiority of sequential add-on therapy for HBsAg reduction in chronic hepatitis B patients with long-term NA therapy has not been settled. Therefore, we contend that this pilot study provides evidence in favour of the superiority of sequential therapy over add-on therapy. According to the changes of HBsAg levels in patients who were treated with add-on therapy, HBsAg levels were not greatly reduced in most patients. We hypothesized that host immune responses might be activated by the cessation of NA therapy and that this activation could help bolster the antiviral effects of PEG-IFN. On the other hand, in patients undergoing add-on therapy, it might be difficult to activate host immunity while undergoing continuous NA therapy, leading to poor reduction of HBsAg. In support of this idea, ALT elevation, which might be induced by the activation of host immunity, occurred in 60% of patients treated with sequential therapy. This ALT elevation was significantly associated with HBsAg reduction by PEG-IFN therapy (Figure 3; P=0.038).

Recently, Murata et al. [25] demonstrated that IFN-\(\lambda\)3 could be induced in colon cancer cell lines by treatment with nucleotide analogues such as ADV or TDF but not by treatment with the nucleoside analogues LMV or ETV. This IFN-\(\lambda\)3 induction was associated with the antiviral effects of sequential therapy with PEG-IFN therapy [24]. To analyse the influence of nucleotide analogue treatment, we compared the effects of PEG-IFN therapy on HBsAg reduction. Although we did not observe differences in HBsAg reduction during and after PEG-IFN therapy (Tables 1 and 2), HBsAg loss was achieved within 3 years after PEG-IFN therapy in 3 of 6 patients who were treated with nucleotide analogues prior to PEG-IFN therapy (data not shown), and this result might indicate the superiority of prior NA treatment for HBsAg loss.

In a recent study, we found that HBV infection induced IL-8 production in hepatocytes and that this IL-8 induction contributes to IFN resistance in human hepatocytes [16]. Therefore, we hypothesized that serum IL-8 regulation might be associated with the antiviral effects of PEG-IFN therapy. We compared serum IL-8 levels between patients with and without \(>0.5\) log IU/ml HBsAg reduction within 1 year after PEG-IFN therapy. As shown in Figure 2, serum IL-8 levels at the end of PEG-IFN therapy in patients who achieved more than 0.5 log IU/ml HBsAg reduction were significantly lower than those in patients with less than 0.5 log IU/ml HBsAg reduction (P=0.044). These results suggest that serum IL-8 reduction during NA and PEG-IFN therapy and the recovery of host immune responses could accompany the reduction of HBsAg levels after PEG-IFN therapy.

In this study, we demonstrated that sequential therapy might be superior to add-on therapy in reducing HBsAg levels in chronic hepatitis B patients undergoing long-term NA therapy. Although the number of study subjects was small, to our knowledge, this is the first study to compare the antiviral effects of sequential versus add-on therapy. Our results support the need for establishing strategies for inducing HBsAg loss in chronic hepatitis B patients.

Acknowledgements

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Disclosure statement

MT – grant/research support: Bristol-Myers Squibb, MI – grant/research support: Bristol-Myers Squibb, KC – speaking and teaching: Sumitomo Dainippon
Additional files

Additional file 1: A table showing the clinical background of study subjects can be found at https://www.intmedpress.com/uploads/documents/4295_Tatsukawa_Addfile1.pdf

Additional file 2: A figure showing reduction of HBsAg titres during PEG-IFN treatment can be found at https://www.intmedpress.com/uploads/documents/4295_Tatsukawa_Addfile2.pdf

Additional file 3: A figure showing the association between ALT flare during PEG-IFN treatment and serum IL-8 levels at the beginning and at the end of PEG-IFN therapy can be found at https://www.intmedpress.com/uploads/documents/4295_Tatsukawa_Addfile3.pdf


References


