PEG-IFN α /RBV Combination Therapy for Chronic Hepatitis C Patients Increases Serum Ferritin Level while It Improves Sustained Viral Response Rate

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Key Words

Alanine aminotransferase • Chronic hepatitis C • Combination therapy • Hemolytic anemia • Hepatic iron overload • Pegylated interferon • Ribavirin • Serum ferritin • Sustained viral response

Abstract

Objectives: We investigated the significance of serum ferritin levels in pegylated interferon (PEG-IFN) and ribavirin (RBV) combination therapy for chronic hepatitis C (CHC) and examined its correlation with serum alanine aminotransferase (ALT) levels during therapy and response to the therapy. **Methods:** A total of 175 patients with CHC received the combination therapy. Correlations between serum ferritin levels and serum ALT levels at 12 and 24 weeks of therapy were examined. Differences in serum ferritin levels during therapy between patients with sustained viral response (SVR) and non-SVR were also examined. **Results:** Only 24 (13.7%) and 20 (11.4%) patients showed elevated serum ALT levels (≥ 70 IU/l) at 12 and 24 weeks of therapy, respectively. There was no correlation between serum ferritin levels and ALT levels. Ninety-five (54.3%) of 175 patients achieved SVR. Serum ferritin levels increased dramatically in both SVR and non-SVR groups after starting the therapy and were significantly higher in the SVR group throughout the therapy. **Conclu-**

sions: Serum ferritin level increases during PEG-IFN and RBV combination therapy; however, it did not correlate with either serum ALT level or the total dose of RBV. Higher serum ferritin levels during combination therapy appear to be associated with favorable therapeutic response.

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Introduction

Pegylated interferon (PEG-IFN) and ribavirin (RBV) combination therapy is the current standard treatment for chronic hepatitis C (CHC) infection, demonstrating an improved sustained viral response (SVR) rate even in patients infected with hepatitis C virus (HCV) genotype 1 and who had a high viral load [1, 2]. Several host and viral factors contribute to SVR in the combination treatment for Japanese patients infected with HCV genotype 1 with high viral load [3–7]. The host factors include younger age, male gender, mild liver fibrosis, platelet count, LDL cholesterol values and γ -glutamyl transpeptidase values. The viral factors include amino acid substitutions in the IFN sensitivity-determining region of the HCV nonstructural 5A (NS5A) protein and in the HCV core region.

Hepatic iron overload is frequently observed in patients with HCV infection and has been considered to be associated with disease progression and hepatocarcinogenesis [8, 9]. However, the association between hepatic iron overload and therapeutic response to IFN therapy for CHC remains controversial [10–12]. We previously reported that elevation of serum alanine aminotransferase (ALT) levels during PEG-IFN α -2a monotherapy for CHC seems to be caused by hepatic iron overload which may be induced by the therapy itself [13].

In this study, we investigated the correlation between serum ferritin levels and serum ALT levels during PEG-IFN and RBV combination therapy for CHC, and also investigated whether the elevated serum ferritin level during therapy is associated with a therapeutic response.

Patients and Methods

Patients

This retrospective study was conducted at the Kinki University of Medical Science. Eligible subjects were: CHC patients who had received weekly injections of PEG-IFN α -2a or -2b for 24–72 weeks; who had been followed for more than 24 weeks after treatment, and who had been examined serially for quantitative and qualitative HCV RNA, serum ferritin, serum iron, ALT and complete blood cell counts.

Patients with a high load (≥ 100 kIU/ml) of HCV genotype 1 were treated for 48–72 weeks, those with a low load (< 100 kIU/ml) of genotype 1 for 24–48 weeks and those with genotype 2 were treated for 24 weeks. PEG-IFN α -2a was administered once a week at a daily dose of 90 or 180 μ g, PEG-IFN α -2b was administered once a week at a daily dose of 1.5 μ g/kg body weight. RBV was orally administered daily in 2 divided doses. The doses of RBV were adjusted based on the subject's body weight (600 mg for ≤ 60 kg, 800 mg for 60–80 kg, 1,000 mg for ≥ 80 kg). Doses were adjusted during therapy according to standard indications.

Patients with hepatitis B virus infection, HIV infection, autoimmune hepatitis, primary biliary cirrhosis, alcoholic liver disease, non-alcoholic steatohepatitis, hemochromatosis, Wilson's disease and hemoglobinopathy were excluded. Patients were classified as responders if they achieved SVR (defined as undetectable HCV RNA at 24 weeks after the completion of therapy). The remaining patients were categorized as non-SVR. Written informed consent was obtained from all patients before treatment and the protocol was approved by the ethics committee of the Kinki University School of Medicine.

Biochemical and Virological Assay

Laboratory tests including serum ALT levels, serum iron, serum ferritin and complete blood cell counts were assessed in a centralized laboratory using automated methods.

Quantitative HCV testing was performed using the Cobas® Amplicor HCV Monitor Test v.2.0 (Roche Diagnostics, Australia) on the Roche Cobas Amplicor Analyzer (Roche Diagnostics) ac-

Table 1. Demographics and baseline characteristics

Patients, n	175
Age	60.2 $\pm$ 0.8
Sex (male/female), n	85/90
BMI, kg/m ²	22.9 $\pm$ 0.3
Genotype, n	
1	151 (86.3%)
2	24 (13.7%)
Viral load, kIU/ml	2,200 $\pm$ 200
WBC, $\times 10^3/\mu$ l	4.9 $\pm$ 0.1
Hb, g/dl	13.8 $\pm$ 0.1
PLT, $\times 10^4/\mu$ l	16.3 $\pm$ 0.5
Serum ferritin, ng/ml	115.6 $\pm$ 12.5
ALT, IU/l	71.5 $\pm$ 4.3
PEG-IFN, n	
α -2a	42 (24%)
α -2b	133 (76%)

BMI = Body mass index; WBC = white blood cell count; Hb = hemoglobin; PLT = platelets.

cording to the manufacturer's instructions. HCV RNA qualitative determination was performed by real-time PCR on a Cobas TaqMan 48 Analyzer or by Cobas Amplicor HCV Test, v.2.0 (Roche Diagnostics) on the Roche Cobas Amplicor Analyzer with a sensitivity limit of at least 50 IU/ml. Laboratory tests and HCV RNA were analyzed before treatment and at 4, 12 and 24 weeks after initiation of therapy, at the end of the treatment period and 4, 12, 24 and 48 weeks following completion of therapy.

Statistical Analysis

Data were expressed as the means $\pm$ standard errors. Differences between groups were determined by Wilcoxon's signed rank test and confirmed by the non-parametric Mann-Whitney U test between groups. Correlation between data was tested using the non-parametric Spearman rank correlation analysis. Differences were considered statistically significant at $p < 0.05$. Statistical calculations were performed using the commercially available software SPSS v.11.5 (SPSS, Chicago, Ill., USA).

Results

Demographics and Baseline Features

Of the 187 patients, 12 discontinued treatment due to adverse events. The remaining 175 patients, consisting of 90 (51.4%) women and 85 men (48.6%), who met the requirements were enrolled in the study. HCV genotype 1 was prevalent in 151 (86.3%) patients with the remaining 24 (13.7%) positive for type 2. PEG-IFN α -2b was administered to 133 (76%) of the patients (table 1).

Table 2. Univariate analysis for SVR

	SVR	Non-SVR	p value
Patients, n	95	80	
Age, years	60.2 ± 1.2	60.1 ± 9.0	0.418
Sex (male/female), n	50/45	35/45	0.243
BMI, kg/m ²	22.6 ± 4.4	23.2 ± 0.3	0.173
Genotype			<0.05
1	74 (49.0%)	77 (51.0%)	
2	21 (87.5%)	3 (12.5%)	
Viral load, kIU/ml	1,700 ± 200	2,700 ± 400	<0.05
WBC, × 10 ³ /μl	5.1 ± 0.2	4.6 ± 0.2	<0.05
Hb, g/dl	14.0 ± 0.1	13.6 ± 0.2	<0.05
PLT, × 10 ⁴ /μl	17.8 ± 0.6	14.6 ± 0.7	<0.05
Serum ferritin, ng/ml	135.9 ± 14.6	174.1 ± 19.9	0.214
ALT, IU/l	73.6 ± 6.5	69.0 ± 5.3	0.494
PEG-INF			0.321
α-2a	20 (47.6%)	22 (52.4%)	
α-2b	75 (56.4%)	58 (43.6%)	

BMI = Body mass index; WBC = white blood cell count; Hb = hemoglobin; PLT = platelets

Viral Response

SVR was achieved by 95 (54.3%) of the 175 patients, with the remaining 80 patients not responding even at 24 weeks after completion of the treatment. Of the 95 patients achieving SVR, 50 (52.6%) were male and 45 (47.4%) were female. There were no significant differences in the rate of SVR between the 2 sexes ($p = 0.243$). For HCV genotype 2, 21 (87.5%) of 24 patients exhibited SVR. Only 74 (49.0%) of 151 patients infected with HCV genotype 1 demonstrated SVR. Univariate analysis showed that factors significantly associated with SVR were low viral load, elevated white blood cell count, high hemoglobin and high platelet count. Serum ferritin levels tended to be lower in patients with SVR. There was no significant difference in age, sex, body mass index, serum ferritin level and serum ALT level between SVR and non-SVR patients (table 2).

Correlation between Serum ALT and Serum Ferritin Level

Only 24 (14%) and 20 (11%) patients showed elevated serum ALT levels (≥ 70 IU/l) at 12 and 24 weeks of the therapy, respectively. Among the subjects whose ALT levels were ≥ 70 IU/l at weeks 12 and 24, the relationship between the serum ALT and ferritin levels was investigated using Pearson's correlation coefficient test. There

Fig. 1. Serum ferritin levels during and after treatment in patients with or without a SVR. Changes over time of serum ferritin levels in SVR and non-SVR patients after antiviral therapy are displayed. BL = Baseline; ET = end of treatment. * $p < 0.05$, comparing between groups at each time point.

was no significant relation between serum ALT and ferritin levels at each time point ($p = 0.838$, $r = -0.049$ at week 12; $p = 0.142$, $r = 0.340$ at week 24).

Dynamics of Serum Ferritin Level during and after Treatment

Serum ferritin levels increased and peaked between 4 and 12 weeks after commencement of therapy, remained high until the end of the treatment period, and returned to baseline levels after completion of the treatment. If SVR did not occur, serum ferritin levels increased again following completion of therapy (fig. 1). The 'increasing ratio of serum ferritin' was calculated as the ratio of serum ferritin level to baseline serum ferritin level. The increasing ratio of serum ferritin in patients with a SVR was significantly higher than that in non-SVR individuals during treatment (fig. 2).

Correlation between Dosage of PEG-IFN or RBV and Increasing Ratio of Serum Ferritin

We analyzed the relationship between administered doses and serum ferritin during the first 4 and 12 weeks of treatment by Pearson's correlation coefficient test. There was no significant correlation between the administered dose of RBV and increasing ratio of serum ferritin ($p = 0.110$, $r = 0.166$ during the first 4 weeks; $p = 0.071$,

Fig. 2. Increasing ratios of serum ferritin during treatment in patients with or without a SVR. The 'increasing ratio of serum ferritin' was calculated as the ratio of serum ferritin level to baseline serum ferritin level. BL = Baseline; ET = end of treatment. * $p < 0.05$, comparing between groups at each time point.

$r = 0.172$ during the first 12 weeks; fig.3). There was also no correlation between PEG-IFN α -2a or -2b and increasing ratio of serum ferritin (PEG-IFN α -2a: $p = 0.856$, $r = 0.037$ at week 4; $p = 0.752$, $r = -0.58$ at week 12; PEG-IFN α -2b: $p = 0.692$, $r = 0.049$ at week 4; $p = 0.243$, $r = 0.132$ at week 12).

Discussion

We previously reported that elevation of serum ALT levels correlated to serum ferritin levels during PEG-IFN α -2a monotherapy for CHC seems to be caused by hepatic iron overload, which may be induced by the therapy itself [13]. In this paper, 28 (44.4%) of 63 patients exhibited elevated ALT levels (≥ 70 IU/l). Also, serum ALT levels were elevated (≥ 70 IU/l) in 24 (13.7%) of 175 patients at week 12, and in 20 (11.4%) of 175 patients at week 24. In our present study, during PEG-IFN and RBV combination therapy, there was no elevation of serum ALT level in almost all patients, and there was no significant correlation between serum ferritin and ALT levels.

RBV, a guanosine analogue which has a broad antiviral spectrum, is known as an immunomodulator inhibiting the viral RNA polymerase, balancing Th1 and Th2 cell responses and acting by direct cytoprotection [14, 15].

Fig. 3. Correlation between administered doses of RBV and increasing ratio of serum ferritin. The 'increasing ratio of serum ferritin' was calculated as the ratio of serum ferritin level to baseline serum ferritin level. **a** Correlation between the mean dose of RBV and increasing ratio of serum ferritin at week 4. **b** Correlation between the mean dose of RBV and increasing ratio of serum ferritin at week 12.

Although HCV RNA levels and hepatic fibrosis scores did not change significantly in patients with CHC who were treated by RBV alone, serum ALT levels and inflammatory features of liver histology were improved [16, 17]. One possible reason why the correlation between serum ALT and serum ferritin levels was not seen in PEG-IFN and RBV combination therapy, distinct from PEG-IFN α -2a monotherapy, is the efficacy of RBV to improve inflammation of the liver as described above.

In this study, whether SVR was reached or not, serum ferritin levels significantly increased after initiation of therapy, peaked at an early stage (4–12 weeks) after initiation and decreased slowly. In SVR patients, serum ferritin levels after treatment were lower than the initial baseline. In non-SVR patients, serum ferritin levels increased again after completion of therapy with a corresponding increase of HCV-RNA load (data not shown). The increased ratio of serum ferritin (serum ferritin/baseline ferritin ratio) in SVR subjects was significantly higher than in non-SVR individuals. A similar report has been previously published, and its results correspond with what we are reporting here [18].

In general, it is thought that an adequate dose of RBV in the first stages of treatment correlates to SVR [19]. It is expected that the more RBV is administered, the more strongly hemolysis occurs and serum ferritin levels rise. In this study, there was no significant correlation between dose of RBV and serum ferritin levels. Even if the same dose of RBV was administered, metabolism and concentration of RBV differ between individuals. Though

we did not investigate parameters involving hemolysis or RBV concentration, the correlation between rising levels of ferritin and the rate of SVR may be related to RBV-induced hemolysis and/or RBV concentration. Ferrara et al. [18] considered that the rise of serum ferritin correlated to RBV-induced hemolysis at earlier phases of treatment, but the correlation between serum ferritin levels and hemolysis is lost at later phases of treatment. Therefore, they considered that elevated serum ferritin at a later phase might be caused by a reactive response from activated macrophages to interferon [18]. Their hypothesis that elevation of serum ferritin level during the PEG-IFN and RBV combination therapy might be caused not only by RBV-induced hemolysis but also by IFN is quite reasonable, because elevation of serum ferritin levels is also observed in PEG-IFN α -2a monotherapy [13]. Further studies are necessary to confirm these hypotheses.

In conclusion, serum ferritin levels increase during PEG-IFN and RBV combination therapy; however, this did not correlate with either serum ALT levels or the total dose of RBV administered. Higher serum ferritin levels during combination therapy appeared to be associated with favorable therapeutic response.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

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