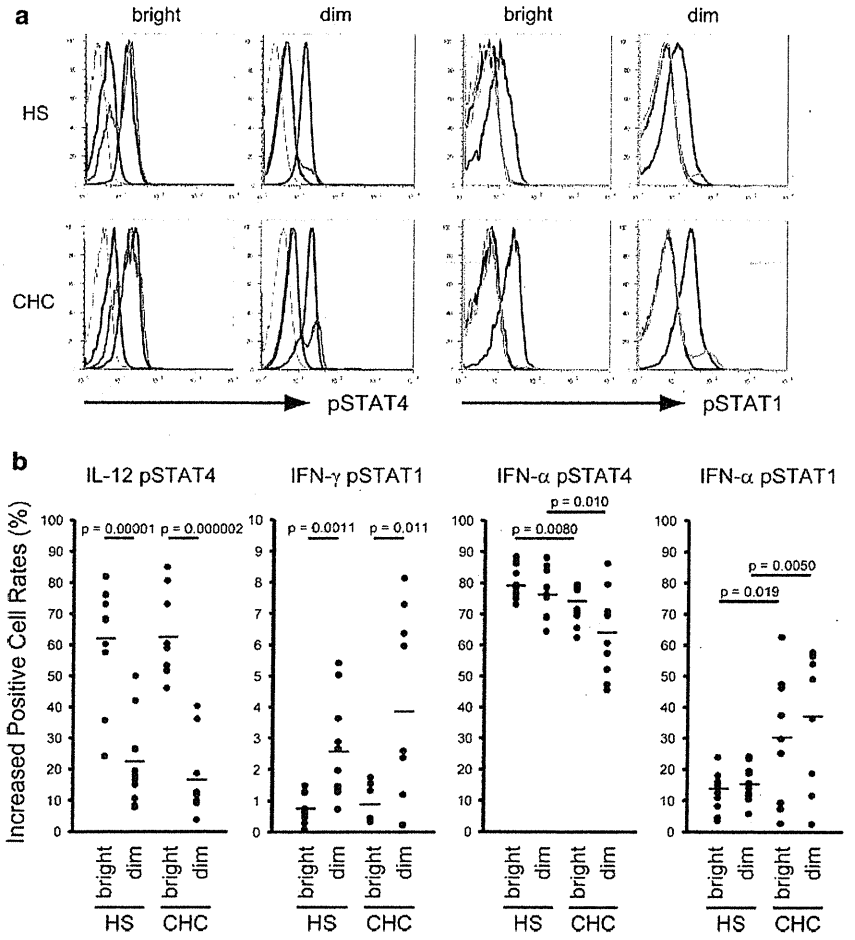

Fig. 4 Activation of STAT1/4 occurring in response to interleukin-12 (*IL-12*), interferon-γ (*IFN-γ*), or IFN-α in NK cell subsets. Phosphorylated STAT1 (*pSTAT1*) and pSTAT4 protein levels were evaluated by flow cytometry with isotype control staining. PBMCs were derived from patients with chronic HCV infection (CHC) ($n = 9$) and healthy subjects (HS) ($n = 11$). Prepared PBMCs were unstimulated or stimulated with natural IFN-α, IFN-γ, or IL-12 for 90 min in vitro, and then collected. pSTAT1 and pSTAT4 protein levels in $CD56^{bright}$ NK (bright) and $CD56^{dim}$ NK (dim) cell subsets were evaluated by flow cytometry, electronically gating on CD56 bright $CD3^-$ cells and CD56 dim $CD3^-$ cells. **a** Representative histograms of a patient and a healthy subject (*HS*) are shown. *Green lines* show staining of IFN-α-stimulated cells with isotype control. *Purple lines* show staining of unstimulated cells with the antibody. *Red, orange,* and *blue lines* show staining of IL-12-, IFN-γ- and IFN-α-stimulated cells, respectively, with the antibody. **b** Positive cell rates were determined based on staining with isotype controls. Increased positive cell rates were determined by subtracting the positive cell rate of unstimulated cells from those of stimulated cells. Comparisons of pSTAT1/4 level in response to IFN-α, IFN-γ, or IL-12 between bright and dim subsets in a subject group or between CHC and HS in a subset are shown as increased pSTAT1/4 positive cell rate with the statistically significant p values. Each *circle* represents individual data. *Horizontal bars* represent means. Statistical significance was analyzed using the unpaired Student's t-test

reported that the hepatic gene expression level in a subset of ISGs, including STAT1, was greater in CHC patients than in normal subjects. Sarasin-Filipowicz et al. [32] showed that the gene expression level in a subset of ISGs in CHC patients was greater in whole liver, including hepatocytes and nonparenchymal cells such as lymphocytes, than in PBMC, and suggested that chronic HCV infection had stronger local effects on the IFN system in the liver than in PBMC. Also, Tateno et al. [33] showed that the gene expression level of STAT1 in liver-infiltrating lymphocytes was about twofold greater than that in hepatocytes in CHC patients. Considering these reports, we speculate that the NK cell subsets in the liver as well as in the peripheral blood of CHC patients might display a high level of STAT1 expression. Whether our findings in peripheral blood could be applied to the liver in CHC patients requires further investigation. We also examined whether our findings with CHC patients would be observed in CHB patients. Unlike in the CHC patients, the CHB patient expression levels of STAT1 in either $CD56^{bright}$ or $CD56^{dim}$ subsets was not significantly higher than that in the HS, which would be consistent with the report of the

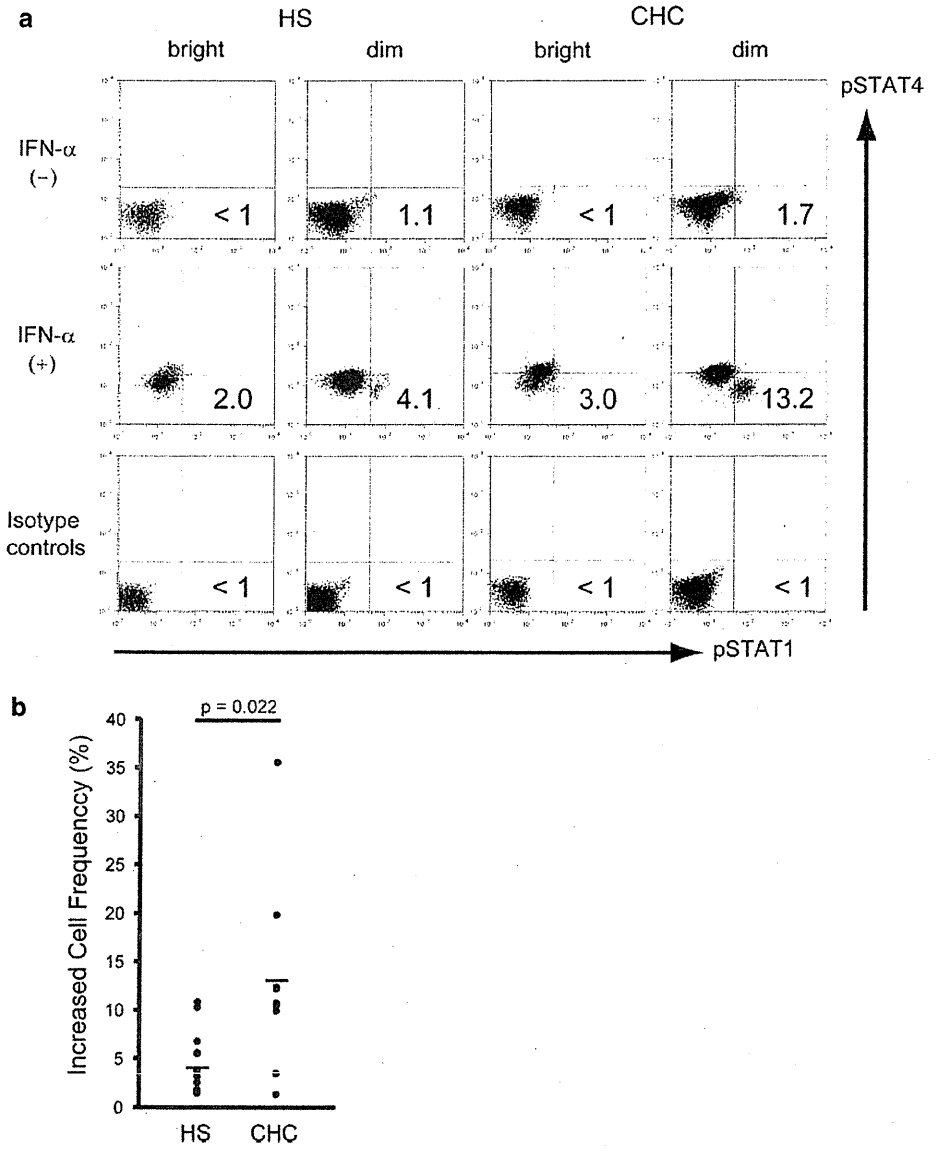

Fig. 5 Relationship between STAT1/4 phosphorylation occurring in response to IFN-α in NK cell subsets. pSTAT1 and pSTAT4 protein levels were simultaneously evaluated by flow cytometry with isotype control staining. PBMCs were derived from patients with chronic HCV infection (CHC) ($n = 9$) and healthy subjects (HS) ($n = 11$). Prepared PBMCs were unstimulated or stimulated with natural IFN-α for 90 min in vitro, and then collected. pSTAT1 and pSTAT4 protein levels in $CD56^{bright}$ NK (bright) and $CD56^{dim}$ NK (dim) cell subsets were evaluated by flow cytometry, electronically gating on CD56 bright $CD3^-$ cells and CD56 dim $CD3^-$ cells. **a** Representative dot plots of untreated or IFN-α treated cells stained with antibody or isotype controls from a patient and a healthy subject (*HS*) are shown. *Numbers* are frequencies of gated cells that strongly phosphorylated STAT1 but weakly phosphorylated STAT4 in the corresponding subsets. **b** Increased cell frequency was determined by subtracting the gated cell frequency of unstimulated cells from those of stimulated cells. Comparisons of the increased cell frequency of the high-pSTAT1 population in response to IFN-α stimulation in the $CD56^{dim}$ NK cell subset between CHC and HS are shown with the statistically significant p value. Each *circle* represents individual data. *Horizontal bars* represent means. Statistical significance was analyzed using the unpaired Student's t-test

STAT1 signaling pathway being less activated in CHB than in CHC [34].

Lines of evidence have shown that $CD56^{dim}$ NK cells, but not $CD56^{bright}$ NK cells, decrease in number in peripheral blood in patients with CHC [12, 14, 15, 35]. In agreement with these reports, we observed a lower frequency of $CD56^{dim}$ NK cells, but not of $CD56^{bright}$ NK cells, in the CHC patients than in the HS (Fig. 1b). Although we observed significant up-regulation of STAT1 expression in both $CD56^{bright}$ NK cells and $CD56^{dim}$ NK cells, the magnitude of the up-regulation of STAT1 expression in the CHC patients, compared with that in the

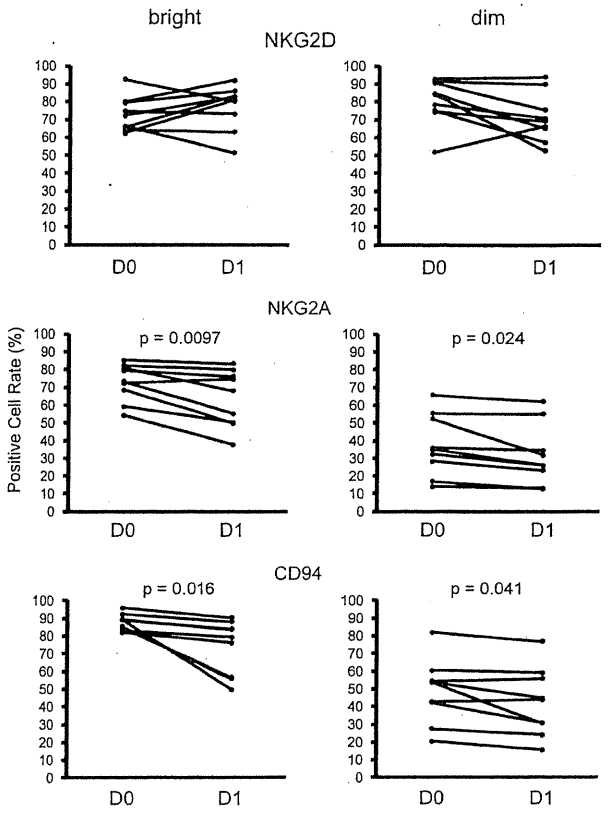

Fig. 6 Regulation of NK receptor expression in response to IFN-α-based therapy. The expression of NK activating or inhibitory receptors, NKG2D or NKG2A and CD94, respectively, on $CD56^{bright}$ NK (bright) and $CD56^{dim}$ NK (dim) cell subsets was evaluated by flow cytometry with isotype control staining, electronically gating on CD56 bright $CD3^-$ cells and CD56 dim $CD3^-$ cells. PBMCs were derived from patients treated with IFN-α-based therapy ($n = 9$) before (*D0*) and 1 day after (*D1*) the initiation of the therapy. Positive cells (positive cell rate) were determined based on isotype control staining. The changes in the NK receptor expression levels between D0 and D1 are shown as positive cell rates with the statistically significant p values. Each *circle* represents individual data. Statistical significance was analyzed using the paired Student's t-test

HS, was clearly greater in $CD56^{dim}$ NK cells than in $CD56^{bright}$ NK cells (Fig. 3b). Considering that STAT1 transmits the anti-proliferative effects induced by IFN-α [36–38], the greater up-regulation of STAT1 in $CD56^{dim}$ NK cells, compared with that of $CD56^{bright}$ NK cells, might have resulted in the significantly reduced frequency in $CD56^{dim}$ NK cells but not in $CD56^{bright}$ NK cells. Further study is required to examine this.

The most prolific producer of IFN-γ is the $CD56^{bright}$ NK cell rather than the $CD56^{dim}$ NK cell [4, 5]. In the present study, we found that $CD56^{bright}$ NK cells responded to IL-12 to phosphorylate STAT4 much more than $CD56^{dim}$ NK cells (Fig. 4a, b). IL-12 is one of the strongest stimulators of IFN-γ production from NK cells, which is transmitted by STAT4 phosphorylation [1, 3, 39, 40]. The preferential activation of STAT4 in $CD56^{bright}$ NK cells, compared with $CD56^{dim}$ NK cells, might be one of the underlying mechanisms by which $CD56^{bright}$ NK cells, compared with $CD56^{dim}$ NK cells, are armed to produce IFN-γ. On the other hand, we found that $CD56^{dim}$ NK cells responded to IFN-γ to phosphorylate STAT1, while $CD56^{bright}$ NK cells hardly did so (Fig. 4a, b). Moreover, some of the $CD56^{dim}$ NK cells responded to IFN-α to more strongly phosphorylate STAT1 than $CD56^{bright}$ NK cells (Fig. 5a). The $CD56^{dim}$ NK cells are strongly cytolytic armed effector cells [4, 5]. IFN-α or IFN-γ is one of the strongest inducers of the cytolytic function of NK cells, which is transmitted by STAT1 phosphorylation [1, 3, 38, 41]. Thus, the predominant activation of STAT1 in $CD56^{dim}$ NK cells, compared with $CD56^{bright}$ NK cells, might be one of the underlying mechanisms by which $CD56^{dim}$ NK cells become armed with a strong cytotoxic function. The differences in cytokine response to activate STAT molecules between these NK cell subsets might lead to the differences in their armed functions, such as cytolysis and cytokine production.

Ahlenstiel et al. [42] have recently reported that chronic exposure to HCV-induced IFN-α rendered NK cells with a functional polarization toward a cytotoxic phenotype, but without an increase in IFN-γ production. Moreover, Oliviero et al. [16] showed that NK cells from CHC patients were of a predominantly activating phenotype and that these phenotypic changes were associated with enhanced cytotoxic activity and defective IFN-γ production. These reports may be associated with our finding that NK cells, including $CD56^{bright}$ and $CD56^{dim}$ subsets, from the CHC patients displayed a high level of STAT1 expression (Fig. 3). Cytotoxic molecules such as perforin and granzyme, as well as STAT1, are among the ISGs [28, 41]. A high level of STAT1 in NK cells, particularly in $CD56^{dim}$ NK cells that are armed with a cytolytic function, in CHC patients might correspond to a high level of cytotoxic molecules in NK cells, resulting in enhanced cytotoxic activity. Indeed, the frequency of the population that strongly phosphorylated STAT1 upon IFN-α stimulation in $CD56^{dim}$ NK cells was significantly higher in the CHC patients than in the HS (Fig. 5b). This population might be highly armed cells with a cytolytic function. On the other hand, it has been reported that the STAT1 expression level in NK cells was correlated negatively with the activation of STAT4 to produce IFN-γ in response to IFN-α in NK cells [22, 24]. A high level of STAT1 in NK cells, particularly in $CD56^{bright}$ NK cells that are armed to produce IFN-γ, might cause defective IFN-γ production in the NK cells of patients with CHC.

Recent studies have demonstrated that a higher level of ISGs in hepatocytes as well as in PBMCs before IFN-α-based therapy is associated with resistance to this therapy

[32, 43]. We have also reported that a small number of CHC patients treated with IFN-α-based therapy revealed a tendency, in those who had a higher level of STAT1 (which is one of the ISGs) in the total NK cell population, to not respond well to the therapy in the early phase, such as in week 8 after its initiation [24]. In the present study, we did not observe a significant correlation between the STAT1 expression level in the NK cell subsets and the sensitivity to IFN-α based therapy, but we did find a tendency of those who had a higher level of STAT1 in the NK cell subsets to not respond well to the therapy in the early phase, such as in week 8 after its initiation (T. Miyagi et al. unpublished data). The number of evaluated patients, however, was small. More data on treated patients will be required to accurately evaluate the relationship between the STAT1 expression level in the NK cell subsets and the therapy outcome.

We have recently reported that NKG2D expression on NK cells could be down-regulated by the soluble major histocompatibility complex class I-related chain A (MICA), which was increased in patients with CHC compared with healthy controls [44]. In the present study, NKG2D expression levels on both $CD56^{bright}$ NK cells and $CD56^{dim}$ NK cells from the CHC patients were significantly lower than those from the HS (Fig. 2). Thus, the lower NKG2D expression on either $CD56^{bright}$ NK cells or $CD56^{dim}$ NK cells in patients with CHC might be caused by the increased soluble MICA. In response to IFN-α treatment in vivo, the expression of NKG2A/CD94 was down-regulated in both subsets in the CHC patients. In vitro stimulation of NK cells with IFN-α did not down-regulate or up-regulate the messenger RNA expression of NKG2A/CD94 in NK cells (T. Miyagi et al. unpublished data). Thus, the lower expression of NKG2A/CD94 might be modulated not directly but indirectly by in vivo IFN-α treatment.

In the present study, we investigated how the NK cell subsets differed in frequency, phenotype, and cytokine response, and also how chronic HCV infection modified these differences. $CD56^{bright}$ NK cells had a relatively higher level of intracellular STAT1 expression than $CD56^{dim}$ NK cells in the HS. Both $CD56^{bright}$ NK cells and $CD56^{dim}$ NK cells from the CHC patients displayed remarkably higher levels of STAT1 expression than those from the HS, without any significant differences between these subsets. Upon in vitro stimulation with cytokines such as IL-12, IFN-γ, and IFN-α, $CD56^{bright}$ NK cells and $CD56^{dim}$ NK cells phosphorylated STAT1/4 differently. These differences between the NK cell subsets in frequency, phenotype, and cytokine response were partly altered in the CHC patients, suggesting their possible association with the persistence of HCV infection and the resistance to IFN-α based therapy. These observations suggest the possibility of cellular or molecular targets for the treatment of chronic HCV infection.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

The efficacy of extended treatment with pegylated interferon plus ribavirin in patients with HCV genotype 1 and slow virologic response in Japan

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Abstract
Background Which patients with hepatitis C virus (HCV) genotype 1 can benefit from extended treatment with pegylated interferon (Peg-IFN) plus ribavirin is unknown, although the overall sustained virologic response (SVR) rate has been shown to improve in patients with a late virologic response (LVR), defined as detectable serum HCV RNA at week 12 and undetectable at week 24.

Methods Among 1163 chronic hepatitis C patients with genotype 1 treated with Peg-IFN plus ribavirin combination therapy, 213 patients with an LVR were examined in this study. In addition, we selected 81 patients of matched sex and age from each of the 48- and 72-week treatment groups, using the propensity score, to compare the efficacy of the two treatment durations.

Results With 72-week treatment, the timing of HCV RNA disappearance and the hemoglobin level at baseline

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showed a strong correlation with the SVR on multivariate analysis. Earlier HCV RNA disappearance was associated with a better SVR rate, regardless of the ribavirin dose (HCV RNA disappearance at week 16, 74%; at week 20, 52%; and at week 24, 31%, $p = 0.01$). The SVR rate with 72-week treatment was higher than that with 48-week treatment, irrespective of age, sex, or the platelet value, and, especially in aged patients (≥65 years old), the SVR rate increased markedly with 72-week treatment (48 weeks, 25% vs. 72 weeks, 56%; $p < 0.05$).

Conclusions An earlier response predicts a higher SVR rate in patients with an LVR given 72-week treatment. Extended treatment with Peg-IFN plus ribavirin for patients with an LVR improved the treatment efficacy, even for aged patients.

Keywords Chronic hepatitis C · Pegylated interferon and ribavirin combination therapy · Extended treatment · Aged patients

Introduction

Long persistence of hepatitis C virus (HCV) infection can lead to the progression of liver fibrosis, causing liver cirrhosis and ultimately hepatocellular carcinoma (HCC) [1, 2]. Past studies have clearly shown alleviation of liver fibrosis, a reduced incidence of HCC, and markedly improved prognosis in patients in whom HCV has been successfully eradicated [3–9]. The currently recommended treatment for chronic hepatitis C is pegylated interferon (Peg-IFN) plus ribavirin therapy, which can improve antiviral efficacy for patients with chronic hepatitis C [10–16]. However, HCV still persists in approximately half of genotype 1 patients treated with Peg-IFN plus ribavirin [12–14, 16]. Accordingly, the treatment method needs to be well managed in order to maximize the virologic response.

For patients with HCV genotype 1, a high sustained virologic response (SVR) rate (73–81%) was found in patients who achieved an early virologic response (EVR), defined as undetectable serum HCV RNA at week 12. However, an SVR was attained at a low rate (14–44%) in patients with a late virologic response (LVR; defined as detectable serum HCV RNA at week 12 and undetectable at week 24), because of a high relapse rate [13, 16–24]. For the treatment strategy, drug dosages and durations of treatment can be modified by considering individual patient situations. We have reported a dose-dependent effect of ribavirin on reducing the relapse rate for patients responding to Peg-IFN plus ribavirin therapy [17, 18]. However, this effect was limited to patients with an EVR and sufficient efficacy was not observed in patients with an LVR, who should be treated not only with a high dose of ribavirin, but also for a longer duration.

For patients with an LVR, previous studies have verified that extended therapy (72-week treatment) can improve the SVR rate (38–60%) compared to standard 48-week therapy (18–36%) by reducing the relapse rate [19, 20]. However, which group of patients with an LVR can benefit from extended therapy remains obscure. In general, in order to clarify the relationship between treatment duration and anti-viral effect, a randomized control trial (RCT) should be conducted in which patients are distributed into standard and extended-therapy groups. However, it is impossible, from an ethical perspective, to conduct an RCT in Japan, because some previous studies have already revealed the usefulness of extended therapy [19–23].

In the present study, we tried to identify the factors associated with SVR in patients with an LVR infected with HCV genotype 1 who received extended treatment. Furthermore, a case-control matched study was conducted in order to compare the effectiveness of the extended treatment with that of the standard treatment of Peg-IFN plus ribavirin therapy.

Patients and methods

Patients

The present study was a retrospective, multicenter trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. Among 1163 chronic hepatitis C patients with genotype 1 treated with Peg-IFN plus ribavirin combination therapy between December 2004 and June 2007, 213 patients with an LVR who completed the therapy with undetectable HCV RNA at the end of the treatment were enrolled in this study. All patients were Japanese, infected with HCV genotype 1, and having a viral load of more than 10^5 IU/ml. The patients with an LVR continued combination therapy for 48 or 72 weeks according to the decision of the investigator at the participating clinical center. The patients treated for 46–52 weeks were classified as the 48-week treatment group and those who were treated for 68–78 weeks were classified as the 72-week treatment group. The baseline characteristics of all patients before matching are summarized in Table 1. In addition, we selected 81 patients of matched sex and age, using propensity scores, from each of the 48- and 72-week treatment groups.

Patients eligible for this study were negative for hepatitis B surface antigen and anti-human immunodeficiency virus. Patients were excluded from this study if they had decompensated cirrhosis or other forms of liver disease (alcoholic liver disease, autoimmune hepatitis). This study was conducted according to the ethical guidelines of the

Table 1 Baseline characteristics of patients with LVR according to treatment duration

Factor	48 weeks	72 weeks	*p* value
Number of patients	106	107	
Age (years)	56.6 ± 9.1	60.2 ± 7.8	0.002
Sex: male/female	51/55	38/69	0.07
Body weight (kg)	59.9 ± 11.5	59.2 ± 10.3	0.64
History of IFN treatment: naïve/experienced	64/42	69/38	0.57
White blood cells (/mm^3)	4908 ± 1389	4893 ± 1430	0.91
Neutrophils (/mm^3)	2455 ± 936	2503 ± 1042	0.91
Red blood cells (×10^4/mm^3)	438 ± 49	439 ± 38	0.48
Hemoglobin (g/dl)	13.9 ± 1.5	13.9 ± 1.4	0.76
Platelets (×10^4/mm^3)	17.0 ± 5.8	16.2 ± 5.7	0.21
AST (IU/l)	56 ± 34	56 ± 34	0.74
ALT (IU/l)	70 ± 50	68 ± 56	0.78
Serum HCV RNA (KIU/ml)[a]	1850	2400	0.03
Histology (METAVIR)[b]			
Fibrosis, 0–2/3–4	75/7	62/17	0.03
Activity, 0–1/2–3	49/33	45/34	0.75
Peg-IFN dose (μg/kg/week)[c]	1.47 ± 0.17	1.48 ± 0.7	0.21
Ribavirin dose (mg/kg/day)[c]	11.3 ± 1.7	11.5 ± 1.5	0.22
HCV RNA negativity: 16/20/24 weeks[d]	65/23/12	51/32/14	0.23

LVR late virologic response, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *IFN* interferon, *HCV* hepatitis C virus

[a] Data shown are median values

[b] 52 missing

[c] Initial dose

[d] The times of HCV RNA negativity were unknown in 6 patients with 48-week treatment and 10 patients with 72-week treatment

Declaration of Helsinki amended in 2008, and informed consent was obtained from each patient.

Treatment

All patients received Peg-IFN alfa-2b (Pegintron; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (Rebetol; Schering-Plough) for the duration of the study of 48 or 72 weeks. Peg-IFN alfa-2b was given subcutaneously once weekly at a dosage of 60–150 μg/kg based on body weight (body weight 35–45 kg, 60 μg; 46–60 kg, 80 μg; 61–75 kg, 100 μg; 76–90 kg, 120 μg; 91–120 kg, 150 μg) and ribavirin was given orally twice a day at a total dose of 600–1000 mg/day based on body weight (body weight <60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 1000 mg), according to a standard treatment protocol for Japanese patients.

Dose reduction

Dose modification followed, as a rule, the manufacturer's drug information according to the intensity of the hematologic adverse effects. The dose of Peg-IFN alfa-2b was reduced to 50% of the assigned dose if the white blood cell (WBC) count declined to <1500/mm^3, the neutrophil count declined to <750/mm^3 or the platelet (Plt) count declined to <8 × 10^4/mm^3, and the agent was discontinued if the WBC count declined to <1000/mm^3, the neutrophil count declined to <500/mm^3, or the Plt count declined to <5 × 10^4/mm^3. Ribavirin was also reduced, from 1000 mg to 600 mg, or from 800 mg to 600 mg, or from 600 mg to 400 mg, if the hemoglobin (Hb) level decreased to <10 g/dl, and it was discontinued if the Hb level decreased to <8.5 g/dl. Both Peg-IFN alfa-2b and ribavirin had to be discontinued if there was a need to discontinue one of the drugs. During this therapy, no iron supplements or hematopoetic growth factors, such as erythropoietin alfa or granulocyte–macrophage colony stimulating factor, were administered.

Virologic assessment and definition of virologic response

The serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 KIU/ml; Roche Diagnostics, Branchburg, NJ, USA) and qualitatively analyzed using the

COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/ml). LVR was defined as detectable serum HCV RNA at treatment week 12 and undetectable at treatment week 24; SVR was defined as the absence of detectable serum HCV RNA at 24 weeks after the the end of the treatment, and relapse was defined as the absence of detectable serum HCV RNA at the end of the treatment but detectable serum HCV RNA at 24 weeks after the end of the treatment.

Statistical analysis

Baseline data for various demographic, biochemical, and virologic characteristics of the patients were expressed as means ± standard deviation or median values. To analyze the relationship between baseline data and SVR, univariate analysis using the Mann–Whitney *U*-test or the χ^2test, and multivariate analysis using logistic regression analysis were performed. The significance of trends in values was determined with the Mantel–Haenszel χ^2test. A two-tailed p value of <0.05 was considered significant. Statistical analysis was conducted with SPSS version 15.0J (SPSS, Chicago, IL, USA).

Results

Baseline characteristics and efficacy of treatment in patients with LVR according to treatment duration

Table 1 shows the baseline characteristics of the patients with LVR stratified according to treatment duration before matching. The patients given 72-week treatment were significantly older ($p = 0.002$), had higher HCV-RNA ($p = 0.03$), and included many with advanced liver fibrosis (METAVIR fibrosis score 3 or 4) ($p = 0.03$). Those with 72-week treatment tended to include many female patients compared to the patients given 48-week treatment ($p = 0.07$). Drug reductions due to side effects occurred with a higher frequency in the 72-week treatment group than in the 48-week treatment group; Peg-IFN, 48-weeks, 40% (42/106) versus 72-weeks, 55% (59/107); ribavirin, 48-weeks, 53% (56/106) versus 63% (67/107). However, the main reasons for reductions of both drugs were almost the same; Peg-IFN, 48-weeks, neutropenia ($n = 23$), thrombocytopenia ($n = 14$); 72-weeks, neutropenia ($n = 24$), thrombocytopenia ($n = 19$), general fatigue ($n = 4$); and ribavirin, 48-weeks, anemia ($n = 47$), general fatigue ($n = 3$); 72-weeks, anemia ($n = 51$), general fatigue ($n = 4$). The SVR rate with 72-week treatment was significantly higher than that with 48-week treatment (59%, 63/107 vs. 37%, 39/106, $p = 0.002$), due to less relapse after treatment.

Factors associated with SVR for patients with LVR treated for 72 weeks

The baseline factors, including the timing of the HCV RNA disappearance, were assessed for association with SVR by univariate and multivariate logistic regression analyses in the 107 patients with 72-week treatment. Univariate analysis showed that factors significantly associated with SVR were age, sex, red blood cell count, Hb, and the timing of HCV RNA disappearance (Table 2A). The factors selected as significant by univariate analysis were evaluated by multivariate logistic regression analysis. The timing of HCV RNA disappearance and Hb at baseline were independent factors for SVR ($p = 0.002$, $p = 0.002$, respectively) (Table 2B).

Baseline characteristics of matched patients with LVR

In order to reduce the selection bias among the LVR patients with 48- and 72-week treatment, a matched case–control study was performed; 81 patients were selected from each of the two treatment duration groups, by matching sex and age, using propensity scores. Baseline characteristics were about the same for the two groups, except for the red blood cell count and the progression stage of liver fibrosis (Table 3). In terms of age and sex, the mean age of the male patients was 57.2 ± 8.3 years in the 48-week treatment group and 58.5 ± 8.2 years in the 72-week treatment group, and the mean ages of the female patients were 59.9 ± 7.6 and 60.0 ± 8.5 years, respectively. The male–female ratio of patients more than 65 years old was similar for the two treatment duration groups (male/female, 8/16; 48-week treatment, 10/17; 72-week treatment). Those less than 65 years old were of the same proportion (54%, male/female, 26/31; 48-week treatment, 25/29; 72-week treatment).

SVR rate among patients with LVR in relation to the factors at baseline and treatment duration

We analyzed the association between the SVR rate and baseline characteristics using the matched population. The SVR rate with 72-week treatment was significantly higher than that with 48-week treatment regardless of age (<65 years, 72 weeks, 63%, 34/54 vs. 48 weeks, 39%, 22/57, $p = 0.01$; ≥65 years, 72 weeks, 56%, 15/27 vs. 48 weeks, 25%, 6/24, $p < 0.05$) (Fig. 1a). For males, the SVR rate with 72-week treatment was 77% (27/35), which was significantly higher than that with 48-week treatment (38%, 13/34, $p = 0.001$). For females, the SVR rate with 72-week treatment tended to be higher than that with 48-week treatment (72 weeks, 48%, 22/46 vs. 48 weeks, 32%, 15/47, $p = 0.14$) (Fig. 1b). Among female patients

Table 2 Factors associated with SVR among patients with 72-week treatment before matching

Factor	SVR	Relapser	*p* value
A. Univariate analysis			
Number of patients	63	44	
Age (years)	58.8 ± 8.0	62.3 ± 7.2	0.02
Sex: male/female	28/35	10/34	0.03
Body weight (kg)	60.0 ± 10.0	58.2 ± 11.1	0.19
History of IFN treatment: naïve/experienced	38/25	31/13	0.31
White blood cells (/mm^3)	5021 ± 1474	4709 ± 1361	0.22
Neutrophils (/mm^3)	2621 ± 1046	2343 ± 1026	0.15
Red blood cells (×10^4/mm^3)	448 ± 39	426 ± 32	0.005
Hemoglobin (g/dl)	14.3 ± 1.3	13.3 ± 1.2	0.001
Platelets (×10^4/mm^3)	15.8 ± 5.3	16.7 ± 6.3	0.63
AST (IU/l)	56 ± 36	54 ± 32	0.68
ALT (IU/l)	71 ± 62	64 ± 45	0.33
Serum HCV RNA (KIU/ml)[a]	2400	2500	0.88
Histology (METAVIR)[b]			
Fibrosis, 0–2/3–4	34/9	28/8	1.00
Activity, 0–1/2–3	25/18	20/16	0.82
Peg-IFN dose (μg/kg/week)[c]	1.29 ± 0.30	1.28 ± 0.32	0.80
Ribavirin dose (mg/kg/day)[c]	9.7 ± 1.8	9.4 ± 2.1	0.57
HCV RNA negativity: 16/20/24 weeks[d]	39/15/4	12/17/10	0.001

Factor	Category	Odds ratio	95% CI	*p* value
B. Multivariate analysis				
Age	1 year old	–	–	NS
Sex	male/female	–	–	NS
Red blood cells	1 × 10^4/mm^3	–	–	NS
Hemoglobin	1 g/dl	2.030	1.289–3.197	0.002
HCV RNA negativity	16/20/24 weeks	0.751	0.633–0.890	0.001

SVR sustained virologic response, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *CI* confidence interval, *NS* not significant

[a] Data shown are median values

[b] 23 missing

[c] Mean doses throughout the treatment

[d] The times of HCV RNA negativity were unknown in 5 patients with 48-week treatment and 5 patients with 72-week treatment

more than 65 years old, the SVR rate with 72-week treatment increased with marginal significance (72 weeks, 53%, 9/17 vs. 48 weeks, 19%, 3/16, $p = 0.07$).

The SVR rate in patients with no to moderate fibrosis (METAVIR fibrosis score 0–2) was 58% (26/45) among patients with 72-week treatment, and this rate was significantly higher than that among patients with 48-week treatment (35%, 19/55) ($p = 0.03$). On the other hand, for patients with more advanced liver fibrosis (METAVIR fibrosis score 3 or 4), the SVR rate was 54% (7/13) among the patients with 72-week treatment and 33% (1/3) among those with 48-week treatment; the difference was not significant due to the small number of subjects. However, the SVR rate among the patients with a lower Plt value (<12 × 10^4/mm^3 at baseline), which is indicative of advanced fibrosis, was significantly higher among the patients given 72-week treatment (61%, 14/23) than that among those given 48-week treatment (24%, 4/17) ($p = 0.03$) (Fig. 1c).

SVR rate among patients with LVR in relation to the timing of HCV disappearance and treatment duration

We analyzed the association of the SVR rate with the timing of HCV RNA disappearance. The SVR rate among the patients with 72-week treatment was 74% (32/43) in patients with undetectable HCV RNA at week 16, 52% (13/25) at week 20, and 31% (4/13) at week 24, and the rates were higher than those among the patients with 48-week

Table 3 Baseline characteristics of matched patients with LVR

Factor	48 weeks	72 weeks	*p* value
Number of patients	81	81	
Age (years)	58.8 ± 8.0	59.4 ± 8.4	0.52
Sex: male/female	34/47	35/46	1.00
Body weight (kg)	58.9 ± 11.7	60.1 ± 11.0	0.46
History of IFN treatment: naïve/experienced	50/31	48/33	0.87
White blood cells (/mm^3)	4717 ± 1286	5020 ± 1516	0.19
Neutrophils (/mm^3)	2332 ± 926	2611 ± 1133	0.13
Red blood cells (×10^4/mm^3)	433 ± 44	445 ± 35	0.03
Hemoglobin (g/dl)	13.8 ± 1.3	14.1 ± 1.3	0.13
Platelets (×10^4/mm^3)	16.2 ± 5.3	16.2 ± 5.9	0.64
AST (IU/l)	56 ± 35	51 ± 27	0.63
ALT (IU/l)	68 ± 52	61 ± 37	0.88
Serum HCV RNA (KIU/ml)[a]	1900	2400	0.10
Histology (METAVIR)[b]			
Fibrosis, 0–2/3–4	55/5	45/13	0.04
Activity, 0–1/2–3	37/23	41/17	0.34
Peg-IFN dose (μg/kg/week)[c]	1.47 ± 0.19	1.48 ± 0.17	0.31
Ribavirin dose (mg/kg/day)[c]	11.4 ± 1.9	11.5 ± 1.5	0.57
HCV RNA negativity: 16/20/24 weeks	52/18/11	43/25/13	0.34

AST aspartate aminotransferase, *ALT* alanine aminotransferase

[a] Data shown are median values

[b] 44 missing

[c] Initial dose

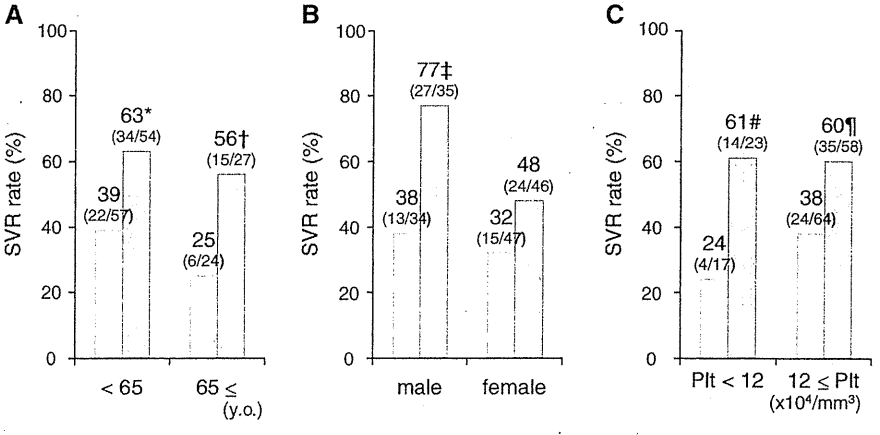

Fig. 1 Sustained virologic response (*SVR*) rate according to baseline characteristics and treatment duration. **a** SVR rate according to age. **b** SVR rate according to sex. **c** SVR rate according to platelet counts. *Light gray shade bars* indicate 48-week treatment. *Dark gray shade bars* indicate 72-week treatment. *y.o.* Years old, *Plt* platelets. $^*p = 0.014$, $^\dagger p = 0.045$, $^\ddagger p = 0.001$, $^\# p = 0.027$, $^\P p = 0.018$ compared to 48-week treatment

treatment (48, 11, and 9%, respectively) (Fig. 2). Regardless of the timing of the HCV disappearance, the SVR rate was raised among the patients with 72-week treatment, and the timing of the HCV RNA disappearance showed a strong correlation with SVR among the patients with 72-week treatment ($p = 0.01$). We also assessed the association of the SVR rate according to ribavirin adherence and the timing of HCV RNA disappearance in LVR patients with each treatment duration (Table 4). Ribavirin adherence was distributed in two categories by mean value (ribavirin throughout the treatment, 9.5 mg/kg/day). Among the patients with 48-week treatment, the SVR rates of patients with higher doses of ribavirin (more than 9.5 mg/kg/day) was slightly higher than that of patients with lower doses of ribavirin (less than 9.5 mg/kg/day) in each of categories of timing of HCV RNA disappearance, but the difference was not significant. However, among the patients given less than 9.5 mg/kg/day, the SVR rate increased significantly in patients with 72-week treatment, compared with 48-week treatment, in patients with

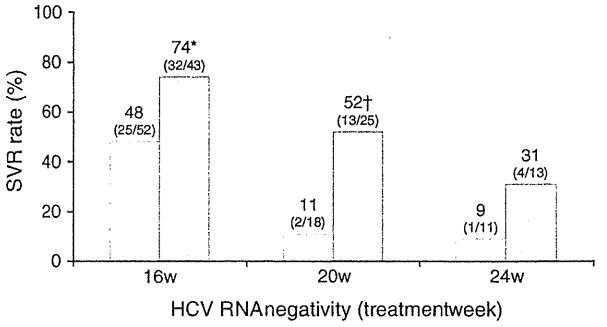

Fig. 2 SVR rate according to timing of hepatitis C virus (*HCV*) RNA negativity. *Light gray shade bars* indicate 48-week treatment. *Dark gray shade bars* indicate 72-week treatment. $^{*}p = 0.012$, $^{\dagger}p = 0.009$, compared to 48-week treatment

Table 4 SVR rate according to timing of HCV RNA negativity and ribavirin adherence

	The timing of HCV RNA disappearance		
	16 weeks	20 weeks	24 weeks
Ribavirin <9.5 mg/kg/day			
48-week treatment	43% (9/21)	0% (0/7)	0% (0/6)
72-week treatment	75% (15/20)	47% (7/15)	25% (1/4)
p value	<0.05	0.05	0.40
Ribavirin ≥9.5 mg/kg/day			
48-week treatment	52% (16/31)	18% (2/11)	20% (1/5)
72-week treatment	74% (17/23)	60% (6/10)	33% (3/9)
p value	0.10	0.08	1.00

undetectable HCV RNA at week 16 (72 weeks, 75% vs. 48 weeks, 43%, $p < 0.05$), and increased with marginal significance in patients with undetectable HCV RNA at week 20 (72 weeks, 47% vs. 48 weeks, 0%, $p = 0.05$). Among the patients with undetectable HCV RNA at week 24, a significant difference was not observed because of the small number of patients in this category. For the patients given more than 9.5 mg/kg/day, the SVR rate with 72-week treatment tended to be higher than that with 48-week treatment, although the patient number was too small to reveal a benefit of extended treatment. As indicated above, the efficacy in patients with LVR given lower doses of ribavirin (less than 9.5 mg/kg/day) could be improved not by an increase in ribavirin dosage, but only by a longer treatment duration, irrespective of the category of timing of HCV RNA disappearance.

Discussion

In order to raise the SVR in patients with HCV genotype 1 treated with Peg-IFN plus ribavirin combination therapy, two strategies are possible: one is the use of a higher dose of drugs and the other is a longer duration of therapy. With respect to drug dose, we have reported that Peg-IFN is dose-dependently correlated with EVR, and ribavirin is dose-dependently correlated with relapse in patients with an EVR [17, 18]. On the other hand, among patients with an LVR, maintaining a high dose of ribavirin (>12 mg/kg/day average dose) did not lead to sufficient reduction of the relapse rate [18]. Thus, the SVR rate in patients with an LVR cannot be improved by a dose-increase strategy, and another treatment strategy, a longer duration of therapy, needs to be devised for patients with LVR in order to reduce the relapse rate.

Past studies have reported that extended therapy reduced the relapse rate. However, more consideration is needed to determine which group of patients can attain the desired effect by extended therapy. Eradication of serum HCV RNA is difficult in female or aged patients or patients with advanced liver fibrosis or a lower Plt count [17, 25], and these patients are considered to be mostly those with an LVR. Previously, we reported that patients more than 65 years old with an LVR showed a low SVR rate [25]. Therefore, in the present study, we tried to identify the group of patients for whom the SVR rate could be improved by extended therapy.

The factors associated with SVR in patients with extended therapy were evaluated by univariate and multivariate logistic regression analyses in the present study. As a result, the timing of HCV RNA disappearance was found to be a significant factor affecting SVR. This suggests that the earlier HCV RNA disappeared, the greater the SVR rate for 72-week treatment as well as 48-week treatment. Examination of the impact of ribavirin exposure on the SVR rate in patients with an LVR showed that, even if a high dose of ribavirin were given, the SVR rate did not show a significant increase among the patients with 48-week treatment, as previously reported [18]. However, the present study showed that an increase in the SVR rate was attained among the patients with 72-week treatment in each category of the timing of HCV RNA disappearance, especially in patients with lower doses of ribavirin. A similar result was found on stratified analysis for the timing of HCV RNA disappearance and Peg-IFN adherence (data not shown). That finding indicated that extend treatment is an effective strategy for LVR patients to increase the SVR rates, although the drug doses of Peg-IFN and ribavirin have been reported to affect the SVR rates in patients with an EVR. And the better efficacy of extended treatment was revealed to be limited to only those patients with earlier HCV RNA disappearance; they are good candidates for extended therapy. Further study is needed to determine whether more extended therapy; for example, 96-week treatment, would be effective for patients with later HCV RNA disappearance.

In the group with extended therapy in the present study, the Hb level at baseline was also significantly associated with SVR. We examined the relationship between Hb level at baseline and age and sex. The mean Hb levels at baseline according to age and sex were highest among male patients less than 65 years old (mean Hb, g/dl, male less than 65 years old; 15.2 ± 1.3, male more than 65 years old; 14.5 ± 0.9, female less than 65 years old; 13.5 ± 1.0, female more than 65 years old; 13.4 ± 1.0). The factors of age and sex were not selected as significant by multivariate analysis, but the Hb level at baseline did affect the SVR rate according to age and sex. In fact, among the patients with 72-week treatment in this study, the SVR rate among male patients less than 65 years old tended to be higher (84%, 21/25) than that of male patients more than 65 years old (60%, 6/10, $p = 0.19$), female patients less than 65 years old (45%, 13/29, $p < 0.01$), and female patients more than 65 years old (53%, 9/17, $p < 0.05$).

In this study, stratified analysis according to baseline factors revealed that extended therapy significantly improved the anti-viral effect, irrespective of age, sex, and Plt value. Especially, 48 weeks of standard treatment was insufficient for an anti-viral effect in aged or female patients, while extended therapy could significantly raise the SVR rate. It is of special clinical significance that extended therapy was found to be beneficial for aged patients, many of whom show an LVR. While the efficacy of extended therapy for patients with advanced liver fibrosis could not be proven in this study, it is conceivable that extended therapy could significantly raise the SVR rate in patients with a lower Plt value, which is indicative of advanced fibrosis. Further study is needed to clarify the efficacy of extended therapy for patients with advanced liver fibrosis.

The main limitation of this study is that it was not designed for randomization, and the treatment duration for patients with an LVR was decided by their physicians. Therefore, older female patients with more advanced liver fibrosis, for whom a poor treatment outcome was expected, tended to be treated for a longer period (72 weeks). However, considering the usefulness of extended therapy for patients with LVR reported in studies from the United States and Europe, there was an ethical issue against conducting an RCT in Japan which would have distributed the patients with an LVR into standard or extended therapy groups. Accordingly, we conducted a case-control study matched for age and sex, in order to compare the efficacy of 72-week treatment with that of 48-week treatment. Because it is known to be difficult to treat aged and female patients with HCV genotype 1 [25], these two factors of age and sex were chosen for minimal matching. As a result, the proportion of patients with advanced liver fibrosis (METAVIR fibrosis score 3 or 4) was not compensated for, and the selected patients in the 72-week treatment group included more patients with advanced liver fibrosis (who are difficult to treat) than the selected patients in the 48-week treatment group. Nevertheless, a higher SVR rate was obtained in the 72-week treatment group in comparison with 48-week treatment.

Recently, genetic polymorphism near the IL28B gene has been reported to be associated with the anti-viral effect of Peg-IFN plus ribavirin combination therapy [26–28]. Single-nucleotide polymorphisms (SNPs) of the IL28B gene are related to on-treatment response (rapid virologic response [RVR], EVR) and SVR [29]. However, no significant difference was observed for relapse after treatment between the major and minor types of IL28B SNPs, if HCV RNA disappeared at the same timing of the treatment [29]. Therefore, the same result as that in the present study may have been attained if the factors of IL28B SNPs had been included as evaluable factors. Further study is needed to examine the issue of the involvement of IL28B SNPs in the efficacy of 72-week Peg-IFN plus ribavirin therapy in patients with LVR.

In conclusion, our results have demonstrated that extended therapy for patients with LVR infected with HCV genotype 1 improved the SVR rate in all categories of patients, even for aged patients with an LVR. The timing of HCV RNA disappearance in patients with an LVR was a predictive factor for SVR and this suggests that response-guided therapy may be needed for later responders.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Efficacy of re-treatment with pegylated interferon plus ribavirin combination therapy for patients with chronic hepatitis C in Japan

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Abstract

Background It is still not known which patients with chronic hepatitis C who failed to respond to previous pegylated interferon (Peg-IFN) plus ribavirin therapy can benefit from re-treatment.

Methods Seventy-four patients (HCV genotype 1, $n = 56$, genotype 2, $n = 18$) were re-treated with Peg-IFN plus ribavirin.

Results On re-treatment, the sustained virologic response (SVR) rate was 41% for genotype 1 and 56% for genotype 2. With genotype 1, the factors associated with an SVR were previous treatment response and the serum hepatitis C virus (HCV) RNA level at the start of re-treatment. Patients with a ≥2-log decrease in HCV RNA at week 12 (partial early virologic response, p-EVR) in previous treatment had significantly higher SVR rates than those without these

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decreases ($p < 0.001$); no patient without a p-EVR in the previous treatment attained an SVR with re-treatment (0/16). All patients with <5 $\log_{10}$ IU/ml of HCV RNA at the start of re-treatment attained an SVR (6/6), while only 33% (15/45) of those patients with ≥5 $\log_{10}$ IU/ml of HCV RNA attained an SVR ($p < 0.01$). Among the patients with relapse in the previous treatment, those who attained an SVR on re-treatment required a longer duration of re-treatment than the duration of the previous treatment (re-treatment, 63.8 ± 13.0 weeks vs. previous treatment, 53.9 ± 13.5 weeks, $p = 0.01$).

Conclusions Re-treatment of genotype 1 patients should be limited to patients with a p-EVR in the previous treatment and a low HCV RNA level at the start of re-treatment. In re-treatment with Peg-IFN plus ribavirin, longer treatment duration can contribute to increasing the anti-viral effect.

Keywords Chronic hepatitis C · Pegylated interferon and ribavirin combination therapy · Re-treatment

Introduction

Pegylated interferon (Peg-IFN) plus ribavirin combination therapy can improve anti-viral efficacy and is currently recommended as first-line therapy for chronic hepatitis C. However, hepatitis C virus (HCV) still persists in approximately half of the genotype 1 patients treated with Peg-IFN plus ribavirin [1–4], and the number of patients who fail to achieve a sustained virologic response (SVR) consequently increases over time.

Recently, the addition of a protease inhibitor to Peg-IFN plus ribavirin combination therapy has been reported to improve the anti-viral effect, but this triple therapy increases side effects, especially severe anemia [5–7]. In Japan, HCV carriers are 10–20 years older than those in the United States and European countries, and patients who are ineligible for triple therapy exist in large numbers due to their potential tendency of having anemia. On the other hand, re-treatment with Peg-IFN plus ribavirin is a possible choice, until triple therapy becomes commercially available, for patients who have failed to show an SVR to previous anti-viral therapy, and for patients who are ineligible for triple therapy. As for re-treatment with Peg-IFN plus ribavirin, there have been only a few studies of patients who failed to show an SVR to previous Peg-IFN plus ribavirin [8–11]. Although re-treatment with Peg-IFN plus ribavirin for patients who failed to respond to previous Peg-IFN plus ribavirin is not recommended in the practice guidelines of the American Association for the Study of the Liver (AASLD) [1], there are some patients who respond to re-treatment. However, it remains obscure in which patients eradication of HCV can be successfully attained by re-treatment with Peg-IFN plus ribavirin.

In the present study, we tried to determine which patients could benefit from re-treatment and to identify the factors associated with an SVR in re-treatment.

Patients and methods

Patients

The present study was a retrospective, multicenter trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. This study was conducted with 74 chronic hepatitis C patients (genotype 1, $n = 56$, genotype 2, $n = 18$) who had previously completed Peg-IFN α-2b plus ribavirin combination therapy but had failed to attain an SVR. Patients were excluded from this study if they had decompensated cirrhosis or other forms of liver disease (alcoholic liver disease, autoimmune hepatitis), or coinfection with hepatitis B or anti-human immunodeficiency virus. This study was conducted according to the ethical guidelines of the Declaration of Helsinki amended in 2008, and informed consent was obtained from each patient.

Treatment

For the previous treatment, Peg-IFN α-2b (Pegintron; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (Rebetol; Schering-Plough) was started between December 2004 and January 2008. For re-treatment with Peg-IFN plus ribavirin, Peg-IFN α-2a (Pegasys; Roche, Basel, Switzerland) plus ribavirin (Copegus; Roche) or Peg-IFN α-2b plus ribavirin was started between February 2006 and January 2009. In principle, as a starting dose, Peg-IFN was given once weekly at a dose of 180 μg of Peg-IFN α-2a and 1.5 μg/kg of Peg-IFN α-2b, and ribavirin was given at a total dose of 600–1000 mg/day based on body weight (for genotype 1, body weight <60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 1000 mg; for genotype 2, body weight <60 kg, 600 mg; >60 kg, 800 mg), according to a standard treatment protocol for Japanese patients.

Dose reduction and discontinuance

Dose modification followed, as a rule, the manufacturer's drug information on the intensity of the hematologic adverse effects. The Peg-IFN α-2a and α-2b doses were reduced to 50% of the assigned dose when the neutrophil count fell below 750/mm^3 or the platelet (Plt) count fell below 8×10^4/mm^3, and the agent was discontinued when the neutrophil count fell below 500/mm^3 or the Plt count fell below 5×10^4/mm^3. Ribavirin was also reduced from 1000 to 600, 800 to 600, or 600 to 400 mg when the

hemoglobin (Hb) was below 10 g/dl, and was discontinued when the Hb was below 8.5 g/dl. Both Peg-IFN and ribavirin had to be discontinued if there was a need to discontinue one of the drugs. No iron supplement or hematopoietic growth factors, such as epoietin alpha or granulocyte–macrophage colony stimulating factor (G-CSF), were administered.

Virologic assessment and definition of virologic response

The serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 KIU/ml; Roche Diagnostics, Branchburg, NJ, USA) and qualitatively analyzed using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/ml). A rapid virologic response (RVR) was defined as undetectable serum HCV RNA level at week 4, a partial early virologic response (p-EVR) was defined as more than a 2-log decrease in HCV RNA level at week 12 compared with the baseline, a complete EVR (c-EVR) was defined as undetectable serum HCV RNA at week 12, a late virologic response (LVR) was defined as detectable serum HCV RNA at week 12 and undetectable at week 24, and an SVR was defined as undetectable serum HCV RNA at 24 weeks after the end of the treatment. Relapse was defined as undetectable serum HCV RNA at the end of the treatment but a detectable amount after the end of the treatment. For both the previous treatment and this re-treatment, patients without a p-EVR or without clearance of HCV RNA at week 24 were considered to be showing non-response (NR) and had to stop treatment. A patient who attained HCV RNA negativity during the re-treatment continued to be treated for 48 or 72 weeks according to response-guided therapy and the decision of the investigator at the participating clinical center.

Statistical analysis

Baseline data of the patients are expressed as mean ± SD or median values. In order to analyze the differences between baseline data or the factors associated with SVR, univariate analysis using the Mann–Whitney *U*-test or the χ^2 test was performed. A two-tailed p value of <0.05 was considered significant. The analysis was conducted with SPSS version 15.0J (SPSS, Chicago, IL, USA).

Results

The baseline characteristics of the patients are summarized in Table 1. Of the 56 genotype 1 patients, 32 were relapsers and 24 showed NR to previous treatment. Among the relapsers, 15 had shown a c-EVR (58%, 15/26) and 29 a p-EVR (100%, 29/29) in the previous treatment. Of the 18 genotype 2 patients, 17 were relapsers and one had shown NR to the previous treatment. Among the relapsers, 5 had shown an RVR (42%, 5/12) in the previous treatment. In the previous treatment, all patients had received Peg-IFN α-2b plus RBV combination therapy. There were no significant differences among the baseline characteristics between the previous treatment and the re-treatment in

Table 1 Baseline characteristics of patients and treatment factors in previous treatment and re-treatment

	Genotype 1						Genotype 2	
	All patients		Previous treatment relapsers		Previous treatment non-responders		All patients	
Number of patients	56		32		24		18	
Sex: male/female	32/24		19/13		13/11		11/7	
	Previous treatment	Re-treatment	Previous treatment	Re-treatment	Previous treatment	Re-treatment	Previous treatment	Re-treatment
Age (years)	57.6 ± 9.2	59.5 ± 9.4	57.8 ± 9.0	59.8 ± 9.4	57.3 ± 9.6	59.0 ± 9.5	57.4 ± 9.0	58.4 ± 1.7
White blood cells (/mm^3)	4909 ± 1404	4670 ± 1566	5117 ± 1276	4756 ± 979	4633 ± 1543	4545 ± 2178	5111 ± 1697	4412 ± 1744
Red blood cells (×10^4/mm^3)	435 ± 40	426 ± 52	444 ± 34	437 ± 36	4243 ± 46	412 ± 67	448 ± 36	447 ± 38
Hemoglobin (g/dl)	13.9 ± 1.2	13.5 ± 1.7	14.1 ± 1.1	13.8 ± 1.3	13.7 ± 1.3	13.1 ± 2.1	14.4 ± 1.2	14.2 ± 1.3
Platelets (×10^4/mm^3)	16.5 ± 6.1	17.5 ± 6.9	18.4 ± 6.6	19.1 ± 6.5	14.1 ± 4.4	15.2 ± 6.9	17.5 ± 6.3	16.2 ± 4.9
AST (IU/l)	58 ± 30	60 ± 45	55 ± 31	56 ± 44	61 ± 28	64 ± 47	52 ± 34	34 ± 13
ALT (IU/l)	74 ± 55	77 ± 74	73 ± 65	79 ± 80	74 ± 40	75 ± 66	65 ± 52	34 ± 18
Serum HCV RNA (KIU/ml)	1600	1100	1600	1100	1600	990	1300	690
Peg-IFN type: α2a/α2b	0/56	24/32	0/32	14/18	0/24	10/14	0/18	4/14

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *HCV* hepatitis C virus, *Peg-IFN* pegylated interferon

Table 2 Factors associated with a sustained virologic response (SVR) in re-treatment with Peg-IFN plus ribavirin

Factor	SVR	Non-SVR	*p* value
Number of patients	23	33	
Age (years)	59.5 ± 7.6	59.5 ± 10.5	0.55
Sex: male/female	16/7	16/17	0.17
White blood cells (/mm^3)	4778 ± 1022	4589 ± 1884	0.29
Neutrophils (/mm^3)	2446 ± 849	2291 ± 1486	0.21
Hemoglobin (g/dl)	13.6 ± 1.3	13.4 ± 1.9	0.73
Platelets (×10^4/mm^3)	18.2 ± 6.3	16.9 ± 7.3	0.28
AST (IU/l)	52 ± 33	65 ± 52	0.46
ALT (IU/l)	75 ± 61	79 ± 82	0.72
Serum HCV RNA: <5log/5log≤	6/15	0/31	<0.01
Peg-IFN type: α2a/α2b	7/16	17/16	0.27
Peg-IFN dose (μg/kg/week)			
α2a	2.64 ± 0.61	2.73 ± 0.72	0.90
α2b	1.18 ± 0.43	1.19 ± 0.34	0.90
Ribavirin dose (mg/kg/day)	8.6 ± 2.9	9.4 ± 2.7	0.28
1st treatment virologic response			
p-EVR; +/−	22/0	14/16	<0.001
Relapse/NR	20/3	12/21	<0.001

p-EVR partial early virologic response, *NR* non-response

terms of peripheral blood cell counts, or the levels of aminotransaminases and serum HCV RNA at the start of treatment.

In genotype 1 patients, the HCV RNA negative rate on re-treatment was 54% (29/54) at week 12 and 71% (40/56) at week 24, and the SVR rate was 41% (23/56). The factors associated with SVR were assessed by univariate analysis for the following variables; age, gender, peripheral blood cell counts, aminotransferases, previous treatment response, serum HCV RNA level, the type of Peg-IFN in re-treatment, and drug adherence (Table 2). As a result, the factors of previous treatment response and serum HCV RNA level at the start of re-treatment were selected as being significant. In examining the efficacy of the re-treatment according to the previous treatment response, the relapsers in the previous treatment had a significantly higher HCV RNA negative rate at weeks 12 and 24 and a significantly higher SVR rate than those with NR in the previous treatment (Fig. 1a). Patients with a p-EVR in the previous treatment showed similar results, while no patient without p-EVR in the previous treatment attained an SVR on re-treatment (0/16) (Fig. 1b). Even among the patients without HCV RNA negativity in the previous treatment, if p-EVR had been attained in the previous treatment, 43% (3/7) of these patients attained an SVR on re-treatment. As for the serum HCV RNA level at the start of re-treatment, all patients with less than 5 $\log_{10}$ IU/ml of HCV RNA attained an SVR (6/6), and 33% (15/45) of those patients with more than 5 $\log_{10}$ IU/ml of HCV RNA attained an SVR ($p < 0.01$).

In examining the efficacy of re-treatment according to treatment duration, among the patients with c-EVR and without RVR on re-treatment, those who were re-treated for 72 weeks tended to attain higher SVR rates than those who were re-treated for 48 weeks (72 weeks, 75%, 9/12, vs. 48 weeks, 25%, 2/8, $p = 0.06$). On the other hand, 43% (3/7) of the patients with an LVR on re-treatment attained an SVR on re-treatment. Among the patients with relapse

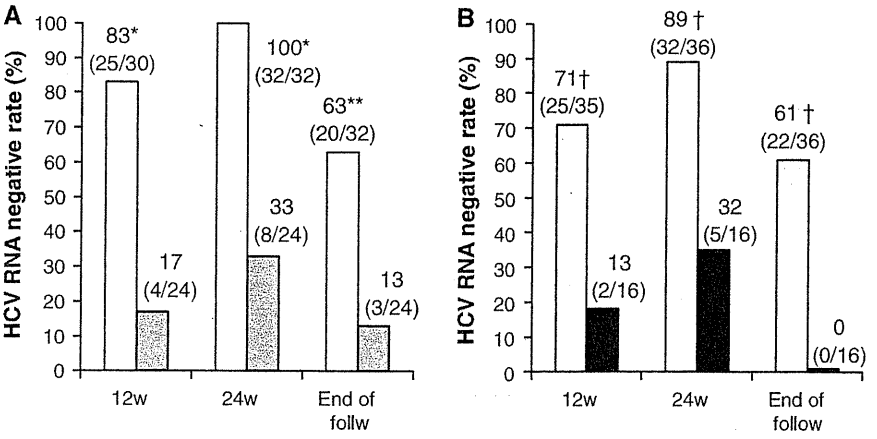

Fig. 1 Virologic response on re-treatment according to previous treatment response. **a** Hepatitis C virus (HCV) RNA negative rate on re-treatment according to relapse or non-response in previous treatment. **b** HCV RNA negative rate on re-treatment according to partial early virologic response (p-EVR) or non-p-EVR in previous treatment. *White bars* patients with relapse in previous treatment. *Dark gray bars* patients with non-response in previous treatment. *Light gray bars* patients with p-EVR in previous treatment. *Black bars* patients with non-p-EVR in previous treatment. *$p < 0.0001$; **$p < 0.01$; compared to non-response. †$p < 0.001$; compared to patients without p-EVR

in the previous treatment, those who attained an SVR on re-treatment required a longer duration of re-treatment than the duration of the previous treatment (re-treatment, 63.8 ± 13.0 weeks vs. previous treatment, 53.9 ± 13.5 weeks, $p = 0.01$), while those without an SVR on re-treatment could be treated for almost the same period as that in the previous treatment (re-treatment, 58.8 ± 12.8 weeks vs. previous treatment, 54.2 ± 11.3 weeks, $p = 0.38$).

Comparison of the timing to the first undetectable HCV RNA level in the previous treatment and re-treatment could be carried out in 50 patients; most patients attained HCV RNA negativity on re-treatment earlier or with the same timing as in the previous treatment, and only one patient showed a later timing for re-treatment. The SVR rate on re-treatment was low, at 13% (3/24) among the patients with detectable HCV RNA at week 24 in the previous treatment. Among the 10 patients with HCV RNA negativity on re-treatment with the same timing as that in the previous treatment, an SVR was attained only by the patients who were re-treated for 72 weeks. Among the 23 patients with earlier HCV RNA negativity on re-treatment, an SVR of 61% was attained (14/23). The patients with an RVR on re-treatment attained a high SVR rate (88%, 7/8) regardless of the virologic response in the previous treatment (Fig. 2).

In genotype 2 patients, the HCV RNA negative rate on re-treatment was 56% (10/18) at week 4, 83% (15/18) at week 12, and 89% (16/18) at week 24, and the SVR rate was 56% (10/18). The two patients without a c-EVR in the previous treatment did not attain an SVR on re-treatment. Among the patients with an RVR on re-treatment, the SVR rates were 60% (3/5) in those with 24-week treatment and 100% (5/5) in those with 48-week treatment.

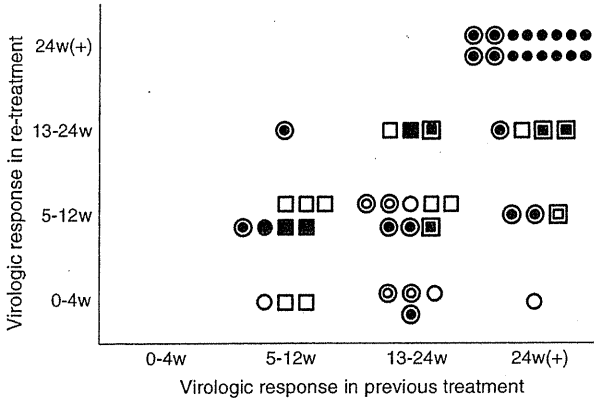

Fig. 2 Virologic response on re-treatment according to the timing of HCV RNA negativity in previous treatment and re-treatment. *Open double circles/open circles* sustained virologic response (SVR) with 48 weeks of re-treatment (*open double circles*, pegylated interferon [Peg-IFN] α-2a plus ribavirin; *open circles* Peg-IFN α-2b plus ribavirin). *Open double squares/open squares*, SVR with 72 weeks of re-treatment (*open double squares* Peg-IFN α-2a plus ribavirin; *open squares*, Peg-IFN α-2b plus ribavirin). *Closed double circles/closed circles*, non-SVR with 48 weeks of re-treatment or non-response (NR) with 24 weeks of re-treatment (*closed double circles*, Peg-IFN α-2a plus ribavirin; *closed circles* Peg-IFN α-2b plus ribavirin). *Closed double squares/closed squares*, non-SVR with 72 weeks of re-treatment (*closed double squares* Peg-IFN α-2a plus ribavirin; *closed squares*, Peg-IFN α-2b plus ribavirin)

Discussion

In the present study of the re-treatment of chronic hepatitis C patients who failed to show an SVR to Peg-IFN plus ribavirin therapy, the patients with relapse in the previous treatment showed a significant response on re-treatment compared with those with NR. This result showed similar findings to the evaluation of peg intron in control of hepatitis C chirrosis (EPIC) study of relapse and NR [10]. In addition, in the present study, p-EVR in the previous treatment was a good indicator of negative prediction for SVR on re-treatment; no patient without p-EVR in the previous treatment attained SVR on re-treatment; that is, the negative predictive value for SVR on re-treatment was 100%. Recently, genetic polymorphism near the IL28B gene has been reported to be associated with the anti-viral effect of Peg-IFN plus ribavirin combination therapy [12–15]. Among Japanese genotype 1 patients, it has been reported that those with the major single-nucleotide polymorphism (SNP) allele of IL28B (rs8099917) show an SVR rate of 39%, while those with the minor allele show an SVR rate of only 11%. Hence, in re-treatment for patients who failed to show a SVR to Peg-IFN plus ribavirin therapy, pretreatment prediction should be done by taking IL28B SNPs and the previous treatment response into account. Patients with the minor SNP allele of IL28B s who did not attain a p-EVR in the previous treatment should wait until new drugs become commercially available.

The next question is how the patients should be re-treated in order to attain an SVR on re-treatment. In the present study, the patients with a low serum HCV RNA level (less than 5 $\log_{10}$ IU/ml) at the start of re-treatment showed a significant rate of cure on re-treatment, and this is almost the same result as that previously reported [9, 10]. In the present study, one patient with NR in the previous treatment started re-treatment with HCV RNA of 52 KIU/ml and attained an RVR and SVR. HCV RNA levels declined on re-treatment among 61% (34/56) of the patients compared to the start of the previous treatment, and it is important not to miss the timing of when the HCV RNA level is low.

With respect to treatment duration among patients with HCV RNA negativity during re-treatment, 72 weeks of treatment tended to increase the SVR rate compared to

48 weeks of treatment (72 weeks, 68%, 15/22, vs. 48 weeks, 44%, 7/16, $p = 0.13$). This result was almost the same as that of the re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha 2b. A randomized trial (REPEAT) study [9]. Furthermore, in the present study, among the patients with relapse in the previous treatment, those who attained an SVR on re-treatment required a longer re-treatment duration than the duration of the previous treatment. In fact, the longer treatment brought about an SVR in some patients whose timing of HCV RNA negativity on re-treatment was the same as that in the previous treatment, as shown in Fig. 2. Thus, especially to be noted is that the relapsers in the previous treatment should be re-treated for a longer period than that of the previous treatment.

It has been reported that splenectomy and partial splenic embolization (PSE) are considered to make it possible for patients with cirrhosis and thrombocytopenia to initiate and continue anti-viral therapy safely, by increasing the platelet counts [16–19]. If poor adherence and inappropriate duration have contributed to a poor response in previous treatment due to thrombocytopenia, there is a possibility that increasing the platelet counts by splenectomy or PSE contributes to improving the tolerability of and adherence to re-treatment, and to increasing the SVR rate in re-treatment. In the present study, one patient with cirrhosis and thrombocytopenia who showed NR in the previous treatment owing to poor adherence to the Peg-IFN α-2b (0.78 μg/kg) regimen underwent splenectomy before re-treatment. As a result, the patient could continue with a sufficient dose of Peg-IFN (1.53 μg/kg) in the re-treatment and attained HCV negativity at re-treatment week 24 and an SVR by extended treatment. Further study is needed on the issue of the effect of splenectomy or PSE in re-treatment on the efficacy of re-treatment with Peg-IFN plus ribavirin therapy.

In the present study, the SVR rate was relatively high (56%) in patients with genotype 2. The patients who could not attain SVR on re-treatment (2 patients) had not attained a c-EVR in the previous treatment. And, among the patients with an RVR on re-treatment, all patients treated for 48 weeks attained an SVR (5 patients), while 40% (2/5) of patients treated for 24 weeks could not attain an SVR. Thus, in patients with genotype 2, as well as in those with genotype 1, the previous treatment response and response-guided therapy can be useful in decisions on the indication for re-treatment or the treatment duration on re-treatment. However, in this study, detailed analysis was not possible because of the small number of genotype 2 patients. Further investigation is needed to clarify this.

The limitation of the present study was that two types of Peg-IFN were used. As for the type of Peg-IFN, some reports have suggested that Peg-IFN α-2a has a stronger anti-viral effect than Peg-IFN α-2b [20, 21], and others have suggested that the two types of Peg-IFN have an almost equal anti-viral effect [22]. In this study, the HCV RNA negative rate at re-treatment week 12 was similar (α-2a, 59%, 13/22, vs. α-2b, 50%, 16/32, $p = 0.51$) between the patients with Peg-IFN α-2a and those with Peg-IFN α-2b. Furthermore, among 24 patients treated with Peg-IFN α-2a on re-treatment, an SVR rate of 38% was attained with 48-week treatment and an SVR rate of 60% was attained with 72-week treatment among patients with a p-EVR in the previous treatment, but no patient without a p-EVR in the previous treatment attained an SVR on re-treatment. Similarly, among 32 patients treated with Peg-IFN α-2b in re-treatment, an SVR rate of 56% was attained with 48-week treatment and an SVR rate of 79% was attained with 72-week treatment among patients with a p-EVR in the previous treatment, but no patient without a p-EVR in the previous treatment attained an SVR on re-treatment. As noted above, since the virologic responses to both Peg-IFNs among re-treated patients were similar, in this study we analyzed the effect of re-treatment without distinction of the type of Peg-IFN.

In conclusion, our results have demonstrated that the efficacy of re-treatment for genotype 1 patients who failed to show an SVR to previous treatment with Peg-IFN plus ribavirin could be predicted by the previous treatment response, especially in terms of p-EVR and a low HCV RNA level at the start of re-treatment. Re-treatment for 72 weeks led to clinical improvement for genotype 1 patients who attained HCV RNA negativity on re-treatment.

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