Research Article

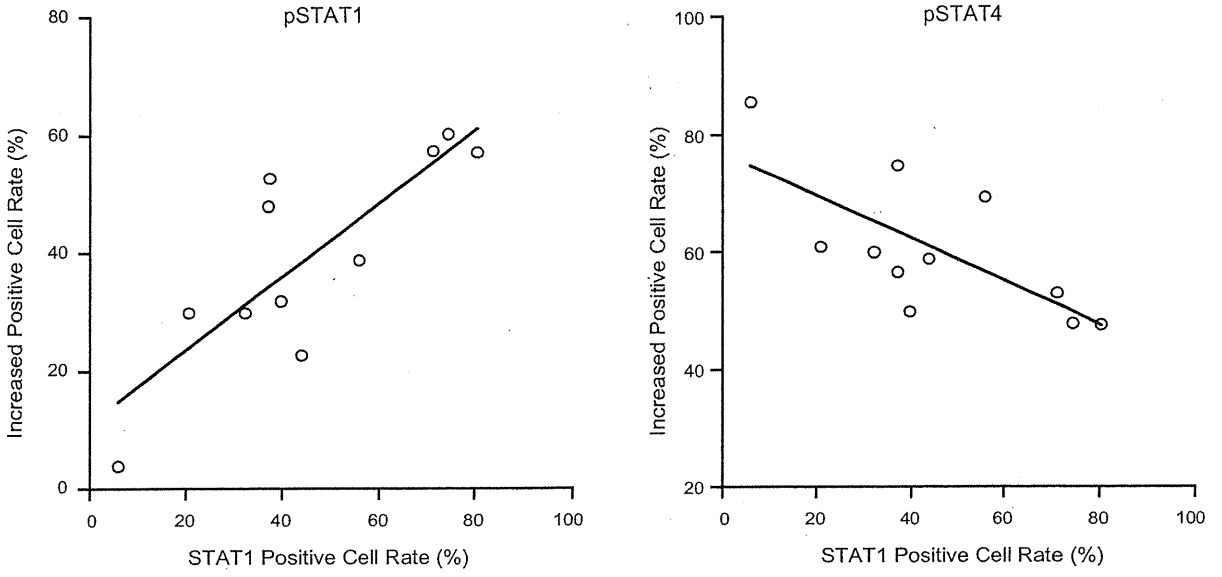

Fig. 4. Correlation between STAT1 level and pSTAT1/4 level in response to IFN-α in NK cells from patients with chronic HCV infection. The relationship was statistically analyzed between intracellular STAT1 level in NK cells and increased pSTAT1/4 level in response to IFN-α in NK cells from patients with chronic HCV infection. Each circle represents individual data. The lines represent regression lines.

which is itself a downstream gene of pSTAT1, by IFN-α based therapy, correlated negatively in vivo with the basal STAT1 expression level before therapy (Miyagi et al. unpublished data). The higher basal STAT1 expression causes a greater level of STAT1 phosphorylation in response to IFN-α, which is followed by a greater level of SOCS1 induction and then might result in a lower increase of STAT1 level. Since perforin and granzyme B are also ISGs from the pSTAT1 pathway [6,25], it is reasonable that the mRNA induction of perforin and granzyme B in response to IFN-α in NK cells from the CHC patients was not significantly greater but modestly lower than those from the HS (Fig. 5). Indeed, the enhancement of degranulation by IFN-α, that is, the increase of CD107a expression, in the presence of K562 cells in response to IFN-α, was significant in NK cells from the HS, but not in those from the CHC patients (Fig. 6).

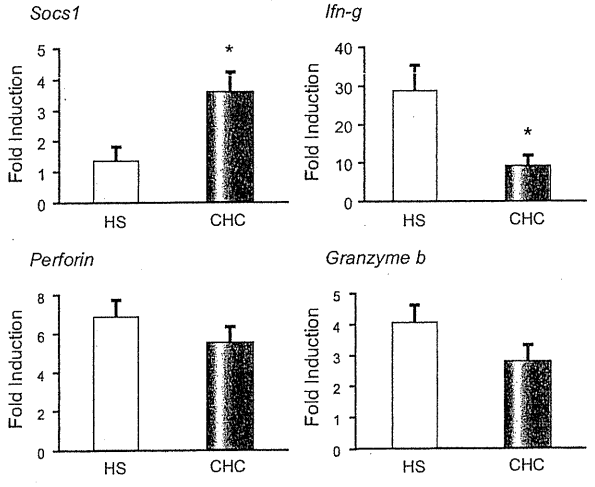

Fig. 5. Induction of ISGs in NK cells in response to IFN-α *in vitro*. NK cells were purified from PBMCs derived from patients with chronic HCV infection (CHC) and healthy subjects (HS). Isolated NK cells were untreated or treated with natural IFN-α *in vitro* for 3 h, and then collected. The collected cell RNA level of the indicated genes and β-actin as a control were analyzed by real-time RT-PCR. Data are shown as the fold increase of treated cells compared with untreated cells, with means ± standard error of the mean from five subjects in each group. *p <0.05 vs. HS.

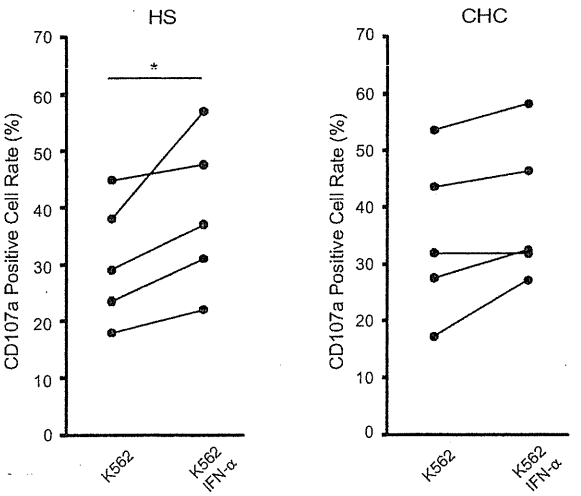

Fig. 6. Increase of NK cell degranulation by IFN-α stimulation. PBMCs derived from patients with chronic HCV infection (CHC) and healthy subjects (HS) were treated with or without IFN-α in the presence of K562 cells. The CD107a expression of NK cells were evaluated by flow cytometry, electronically gating on $CD56^+$ CD3 NK cells. Data are shown as the frequency of CD107a-positive NK cells treated with or without IFN-α. Each circle represents individual data. *p <0.05.

Patients who were clear of HCV due to IFN-α based therapy exhibited a significant increase in NK cell numbers and activity in the peripheral blood as well as in the liver compared to those who were not able to become clear of the virus [26–28]. IFN-γ produced from NK cells have been reported to suppress HCV replication in vitro [29]. IFN-γ from NK cells is also considered to activate the subsequent adaptive immune response during virus infection as well as inhibit viral replication [1,2,7]. These findings suggest the involvement of NK cells with IFN-γ in the response to IFN-α based therapy. The present study showed that the level of STAT4 phosphorylation in response to IFN-α in NK cells correlated negatively with the intracellular STAT1 level in NK cells (Fig. 4), and that IFN-γ induction, which is one of the downstream genes of pSTAT4, was clearly weaker in NK cells from the patients with CHC than in those from the HS (Fig. 5). The lower activation in CHC patients of the pSTAT4-to-IFN-γ pathway in response to IFN-α in NK cells compared to those from the HS might be one of the mechanisms which make CHC patients resistant to IFN-α based therapy.

Ahlenstiel et al. have recently reported that chronic exposure to HCV-induced IFN-α caused NK cells to become functionally polarized towards a cytotoxic phenotype, but that lacked an increase in IFN-γ production [30]. Moreover, Oliviero et al. 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 [31]. These reports would be associated with our finding that NK cells from the CHC patients displayed a high level of STAT1 expression. Cytotoxic molecules such as perforin and granzyme, as well as STAT1, belong to the ISGs [6,25]. A high level of STAT1 in NK cells in the CHC patients might correspond to a high level of cytotoxic molecules in NK cells resulting in enhanced cytotoxic activity at the basal level. Indeed, the CD107a expression at basal level in NK cells from the CHC patients seemed to be modestly higher than that in NK cells from the HS (Fig. 6), although NK cells from the CHC patients did not significantly increase the CD107a expression in response to IFN-α. On the other hand, our present study showed that STAT1 expression level in NK cells correlated negatively with the activation of STAT4 to produce IFN-γ in response to IFN-α in NK cells. A high level of STAT1 in NK cells would also cause defective IFN-γ production in the NK cells of patients with CHC.

Recent studies have revealed that the higher level of ISGs in hepatocytes as well as in PBMCs before IFN-α based therapy is associated with resistance to this therapy [32–33]. The present study demonstrated that NK cells from CHC patients displayed higher levels of STAT1, which is one of the ISGs, compared with those from HS (Fig. 2). Those who had a higher STAT1 level in NK cells showed less STAT4 phosphorylation and more STAT1 phosphorylation in response to IFN-α, resulting in less IFN-γ induction and more SOCS1 induction. Thus, it is possible that those who have a higher STAT1 level in NK cells would display less response to IFN-α based therapy. Our preliminary data with a small number of patients treated with IFN-α based therapy revealed a tendency for those who had a higher STAT1 level in NK cells to not respond well to the therapy in the context of HCV clearance by week 8 after initiation of therapy (Supplementary Fig. 3).

In the present study, we found that the expression level of total STAT1, a key molecule of IFN-α signaling, was clearly higher in NK cells from the patients with CHC than in those from the HS. The phosphorylation levels of STAT1 and STAT4 with IFN-α stimulation were altered in NK cells from the CHC patients, compared with those from the HS. The induction of IFN-γ mRNA expression with IFN-α stimulation, which is one of the downstream genes of pSTAT4, was clearly weaker in isolated NK cells from the CHC patients than in those from the HS. The induction of SOCS1 mRNA expression, which is one of the downstream genes of pSTAT1, was clearly stronger. The enhancement of NK cell degranulation, the increase of CD107a expression, in response to IFN-α was significant in the HS, but not in the CHC patients. These results indicate that IFN-α signaling in NK cells is altered in CHC patients, suggesting that this alteration of IFN-α signaling is associated with the persistence of chronic HCV infection and resistance to IFN-α therapy. The basal total STAT1 level in NK cells might enable the prediction of the outcome of IFN-α therapy against HCV infection, and thus could serve as a molecular target for more effective IFN-α based therapy.

Conflict of interests

The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2010.03.018.

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Original Article

Effect of interferon α-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with chronic hepatitis

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Aim: The objective of this study was to elucidate the long-term effects of interferon (IFN)α-2b plus ribavirin combination therapy and to clarify whether this therapy can reduce the incidence of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C.

Methods: A total of 403 patients infected with hepatitis C virus (HCV) were enrolled in a multicenter trial. All patients were treated with a combination of IFN-α-2b plus ribavirin therapy. We examined the incidence of HCC after combination therapy and analyzed the risk factors for liver carcinogenesis.

Results: A sustained virological response (SVR) was achieved by 139 (34%) of the patients. The cumulative rate of incidence of HCC was significantly lower in SVR patients than in non-SVR patients ($P = 0.03$), while there was no difference in the cumulative incidence of HCC between the transient response (TR) group and the no response (NR) group. Cox's regression analysis indicated the following risk factors as independently significant in relation to the development of HCC: age being > 60 years ($P = 0.006$), advanced histological staging ($P = 0.033$), non-SVR to IFN therapy ($P = 0.044$). The cumulative incidence rate of HCC was significantly lower in patients who had average serum alanine aminotransferase (ALT) levels of < 40 IU/L than in those who showed average serum ALT levels of ≧ 40 IU/L after the combination therapy ($P = 0.021$).

Conclusions: These results suggest that the attainment of SVR or continuous normalization of ALT levels after IFN therapy can affect patients apart from HCC development.

Key words: chronic hepatitis C, continuous normalization of ALT, hepatocellular carcinoma, interferon plus ribavirin combination therapy, sustained virological response

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common malignancies in Japan and its incidence has been increasing over the last 30 years. Recently, various treatments such as transcatheter arterial embolization/chemoembolization, radio frequency ablation and hepatic resection have been reported to yield significant improvements in overall patient survival,[1–3] but HCC relapse has thus far been observed in a majority of treated patients due to the highly malignant potential of the liver. In general, approximately 70–80% of Japanese HCC patients are also diagnosed with type C chronic hepatitis or cirrhosis.[4] It has also been shown that the chronic hepatitis C (CHC) liver slowly but steadily progresses to cirrhosis[5,6] and the risk of HCC increases according to the degree of liver fibrosis.[7,8] In this regard, the success of treatment

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for chronic hepatitis C virus (HCV) infection is expected to prevent the patient's liver from progressing to cirrhosis and to reduce the risk of development of HCC. Interferon (IFN) has been proven to be effective in reducing and in eliminating HCV from the circulation; in decreasing serum alanine aminotransferase (ALT) levels; and in improving the histological appearance of the liver in patients with CHC.[9–11] Moreover, it has been demonstrated that IFN monotherapy in CHC patients is associated with reducing the incidence of HCC, especially in those patients who achieved a sustained virological response (SVR).[12–14] Recently, many investigators have reported that combination therapy using IFN-α-2b or pegylated IFN (Peg-IFN) N plus ribavirin is more effective for eradicating HCV than IFN monotherapy.[15–17] However, it has not been accurately evaluated whether or not the combination therapy using Peg-IFN plus ribavirin could reduce HCC development in patients infected with HCV.

In this study, we evaluated the long-term effect of IFN-α-2b plus ribavirin therapy on the incidence of HCC in HCV-infected patients treated with the combination therapy by retrospective examination of the clinical outcomes.

METHODS

Patients

THIS STUDY WAS a multicenter trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum in Japan. A total of 459 patients with HCV infection were treated with a combination of IFN-α-2b (Intron; Schering-Plough Corporation, Kenilworth, NJ, USA) plus ribavirin (Rebetol; Schering-Plough, Auxerre, France) between June 2002 and March 2005. All patients were treated with 6 MU of IFN-α-2b subcutaneously thrice a week and with oral ribavirin daily. Ribavirin was given at a total daily dose of 600 mg for patients who weighed < 60 kg and 800 mg for patients who weighed ≧ 60 kg. Patients who were positive for hepatitis B surface antigen, anti-human immunodeficiency virus antibody or those with other liver diseases (alcoholic liver disease, autoimmune liver disease, etc) were excluded from this study. Also excluded were patients with a history of HCC and those who developed HCC within the first 6 months of the follow-up period after the end of IFN therapy, because of the possibility that microscopic HCC had been present before initiation of the treatment. The remaining 403 patients infected with HCV were enrolled and followed in this study. The observation term was terminated upon the start of the next IFN therapy, such as Peg-IFN plus ribavirin after a combination of IFN-α-2b plus ribavirin therapy. Responses to IFN therapy were divided into the following three groups based on the viral load: sustained virological response (SVR) was defined as the absence of detectable serum HCV-RNA at 24 weeks after completion of IFN therapy. Transient response (TR) was defined as the absence of HCV-RNA from the serum at the end of treatment but detectable at 24 weeks after completion of therapy. Those categorized as having no response (NR) did not meet these criteria.

This study protocol followed the ethical guidelines of the 1975 Declaration of Helsinki, and informed consent was obtained from each patient.

Blood tests

Serum samples were stored frozen at −80°C. HCV-RNA levels were analyzed by quantitative reverse transcription (RT)–PCR assay (Amplicor-HCV version 2.0; Roche Diagnostic Systems, Tokyo, Japan). The lowest detection limit of this assay was 50 IU/mL. All patients were examined for serum HCV-RNA level and underwent hematological and biochemical tests just before therapy, every 4 weeks during treatment and every 12 weeks thereafter until the end of treatment.

Normal serum ALT is defined as < 40 IU/L. In addition, the biological response to IFN therapy was defined based on "the average serum ALT level", which was calculated from all data of ALT levels after completion of IFN therapy.

Histological evaluation

The patients underwent liver biopsies within 6 months before the start of therapy. Histopathological interpretation of specimens was done by experienced liver pathologists who had no clinical information. The histological appearance of the liver sample sections was evaluated according to METAVIR's histological score.[18] Fibrosis stage was evaluated on a scale from 0 to 4.

Diagnosis and follow up of HCC

Ultrasonography was carried out before IFN therapy and every 3 to 6 months during the follow-up period. New space-occupying lesions detected or suspected at the time of ultrasonography were further examined by computed tomography (CT) or hepatic angiography. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings from CT. If no typical image of HCC was observed, fine-needle aspiration biopsy was carried out with the

patient's consent, or the patient was carefully followed until a diagnosis was possible with a definite observation by CT or angiography.

Statistical analysis

Quantitative variables were expressed as mean ± SD. The Kaplan–Meier method was used to calculate the cumulative incidence of HCC. The prognostic relevance of clinical variables and HCC incidence was evaluated by univariate analysis with log–rank test and by multivariate Cox's regression analysis. A value of $P < 0.05$ (two-tailed) was considered to indicate significance. All calculations were performed with SPSS version 15.0J (SPSS, Chicago, IL, USA).

RESULTS

Baseline characteristics in patients treated with interferon therapy

THE BASELINE CLINICAL features of the enrolled patients are shown in Table 1. The mean age of the patients was 55.8 ± 10.9 years, and 64% of the total cases were male. Two hundred and sixty-one patients (73%) were infected with HCV genotype 1 and had a viral load of more than 10^5 IU/ml. Liver biopsy was done for 320 cases and the ratio of patients with severe fibrosis (F3–4) diagnosed by the HAI score was more than 31%. The mean platelet count was 14.8 ± 5.1 × $10^4/\mu l$, and the ALT level was 96.0 ± 62.6 IU/L. A sustained virological response (SVR) was achieved by 139 patients (34%) by combination therapy of IFN-α-2b plus ribavirin. According to an intent-to-treat analysis, 20% (51/261) of patients with HCV genotype 1 and a high viral load (≧ 100KIU/mL) achieved SVR by the combination therapy, whereas 75% (73/97) of the patients with HCV genotype 2 or a low load showed SVR. The median observation period for all patients was 36.5 ± 14.8 months with a range of 6 to 62 months from the end-point of IFN treatment.

Table 1 Baseline characteristics in patients treated with interferon therapy

	All cases
Number of patients	403
Age	55.8 ± 10.9
Gender (male/female)	257/145
Genotype and viral load (1H/non-1H)	261/97
Fibrosis (F0/1/2/3/4)	15/149/56/92/8
WBC ($/\mu l$)	5113 ± 1487
Platelet ($\times 10^4/\mu l$)	14.8 ± 5.1
ALT (IU/l)	96.0 ± 62.6
IFN effect (SVR/TR/NR/cessation)	139/109/110/45

Data are number of patients, mean ± standard deviation. Fibrosis stage is evaluated on a scale from 0 to 4 according to METAVIR's histological score. 1H, Genotype 1 and high viral load; non-1H, all except for 1H; ALT, alanine aminotransferase; IFN, interferon; NR, no response; SVR, sustained virological response; TR, transient response; WBC, white blood cells.

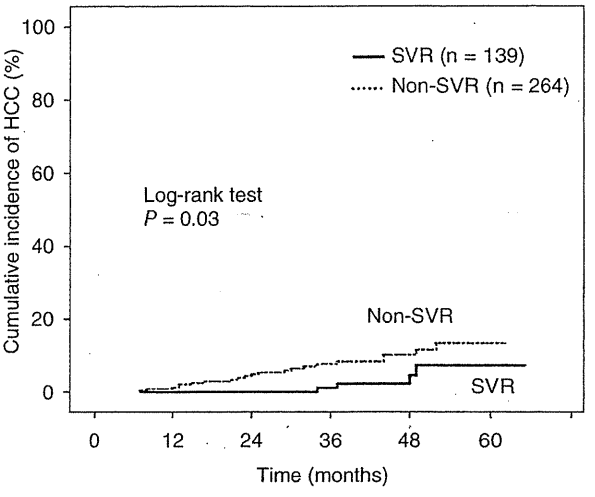

Figure 1 Cumulative incidence of development of hepatocellular carcinoma (HCC) according to treatment effect: (—) sustained virological response; (.....) non-sustained virological response.

Cumulative incidence of development of HCC according to the treatment effect (SVR vs. non-SVR)

Figure 1 shows the Kaplan–Meier estimates of the cumulative HCC incidence according to the treatment effect (SVR vs. non-SVR). Twenty-five (6%) of the 403 enrolled patients developed HCC; four (2.9%) of the SVR group and 21 (8.0%) of the non-SVR group. The cumulative incidence rate of HCC was significantly lower in patients of the SVR group than in those of the non-SVR group ($P = 0.03$).

Cumulative incidence of HCC development according to the treatment effect (SVR vs. TR vs. NR vs. cessation)

Figure 2 shows the Kaplan–Meier estimates of the cumulative HCC incidence according to the treatment effect (SVR vs. TR vs. NR vs. cessation). Five patients (4.6%) of the TR group, nine (8.2%) of the NR group and seven (15.6 %) of the cessation group developed

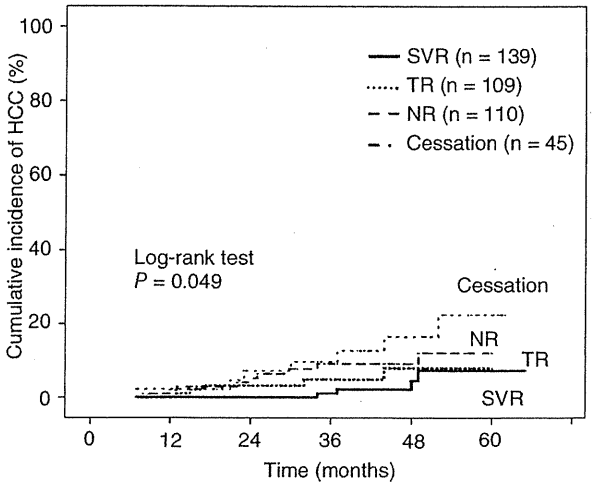

Figure 2 Cumulative incidence of hepatocellular carcinoma (HCC) development according to treatment effect: (—) sustained virological response; (.....) transient response group; (– –) no response; (– ·) cessation.

HCC. There was no significant difference in the cumulative incidence of HCC between the TR and NR groups ($P = 0.394$). In contrast, the cumulative incidence rate of HCC was significantly lower in patients of the SVR group than in those of the NR group ($P = 0.05$). These results indicate that treatment of the TR group with IFN-α-2b plus ribavirin therapy did not reduce HCC development when compared to the NR group.

Risk factors for cumulative incidence of HCC development

Univariate analysis with the log–rank test showed that the following were significant risk factors for the development of HCC; older age (> 65 years) ($P = 0.01$), severe fibrosis ($P = 0.006$), high platelet count ($> 14 \times 10^4/\mu l$) ($P = 0.017$) and non-SVR ($P = 0.03$). Stepwise multivariate analyses of these four variables were performed for all patients treated with combination therapy of IFN-α-2b plus ribavirin by Cox's regression analysis, as shown in Table 2. The analysis indicated the following factors as independent significant risk factors related to the development of HCC: older age (risk ratio, 3.23; 95% CI, 1.37–8.56; $P = 0.006$), fibrosis staging (risk ratio, 1.69; 95% CI, 1.04–2.67; $P = 0.033$) and non-SVR to IFN therapy (risk ratio, 3.57; 95% CI, 1.04–12.36; $P = 0.044$).

Cumulative incidence of HCC development according to average serum ALT levels after combination therapy

The average serum ALT levels in 134 patients (96.4%) of the SVR group were < 40 IU/L after completion of the combination therapy, while 63 patients (24.4%) of the non-SVR group showed serum ALT levels of ≧ 40 IU/L. Figure 3 shows Kaplan–Meier estimates of the cumulative HCC incidence according to the average serum ALT levels after combination therapy. The cumulative incidence rate of HCC was significantly lower in patients with average serum ALT levels of < 40 IU/L than with average serum ALT levels of ≧ 40 IU/L ($P = 0.021$).

Cumulative incidence of HCC development according to the treatment effect (SVR vs. non-SVR) in patients showing less than 40 IU/L average ALT levels after the combination therapy

Figure 4 shows Kaplan–Meier estimates of the cumulative HCC incidence according to the treatment effect (SVR vs. non-SVR) in patients who showed less than 40 IU/L average ALT levels after the combination therapy. There was no significant difference in the cumulative incidence rate of HCC between the SVR and non-SVR groups ($P = 0.37$).

Table 2 Risk factors for cumulative incidence of HCC development

Variable	Category	Risk ratio	*P* value	95% CI
Gender	male	1		
	female	0.34	0.053	0.11–1.01
Age (years)	65 <	1		
	65 ≥	3.23	0.006	1.37–8.56
Fibrosis	F0/1/2/3/4	1.69	0.033	1.04–2.67
IFN therapy	Non-SVR	1		
	SVR	0.28	0.044	1.04–12.36

CI, confidence interval; IFN, interferon; SVR, sustained virological response.

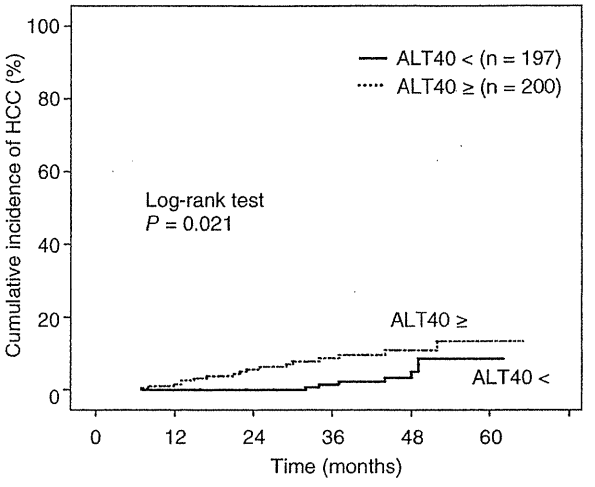

Figure 3 Cumulative incidence of HCC development according to average alanine aminotransferase (ALT) levels after the combination therapy. (—) ALT < 40 IU/ml; (.....) ALT > 40 IU/ml.

DISCUSSION

COMBINATION THERAPIES USING IFN-α-2b or Peg-IFN plus ribavirin have been proven to be more effective in treating for HCV infection than IFN monotherapy.[15–17] However, it has not been accurately evaluated whether the combination therapies using IFN-α-2b or Peg-IFN plus ribavirin could reduce the development of HCC, and what the risk factors of HCC incidence were in patients infected with HCV. In this study, we retrospectively examined the incidence of HCC with IFN-α-2b plus ribavirin therapy to clarify the indicators of combination therapy for reducing HCC in patients infected with HCV. We also evaluated whether or not SVR or continuous normalization of ALT levels could reduce the risk of development of HCC.

Previous studies have demonstrated that IFN monotherapy has a preventive effect on the development of HCC, especially in patients with SVR.[12–14] In this study, using the combination of IFN-α-2b plus ribavirin, we obtained almost the same result for the SVR group treated with IFN-α-2b plus ribavirin therapy, which showed a significantly lower possibility of HCC development over a long-term period when compared with the non-SVR group. In contrast, we found no difference in the cumulative incidence of HCC between the TR and NR groups, while Kasahara *et al.* reported that the cumulative incidence of HCC in patients who achieved TR by IFN monotherapy was significantly lower than those with NR.[13] Recent reports have demonstrated that the combination therapy of IFN-α-2b plus ribavirin is able to induce a SVR in a significant proportion of patients with IFN monotherapy-resistant chronic hepatitis C,[19,20] suggesting that a viral relapse after IFN therapy is efficiently suppressed by combination with ribavirin. Since the combination therapy was a more effective treatment for HCV infection than IFN monotherapy[15–17] and there are fewer TR patients with combination therapy than with monotherapy, we speculate that not all, but quite a few patients of the TR group given IFN monotherapy corresponded to the SVR group given the combination therapy, and that the TR group given the combination therapy might have been included in the NR group of IFN monotherapy. This would mean that the "TR group given combination therapy" should be distinguished from the "TR group given IFN monotherapy", and might explain why the results of this study were inconsistent with previous reports of the cumulative incidence of HCC in the TR group given IFN monotherapy being significantly lower than those with NR.[13]

The Kaplan–Meier method showed that older age (> 65 years), severe fibrosis (F2–4), high platelet count (> 14×10^4) and non-SVR were significantly associated with the development of HCC. The Cox's regression analysis indicated that older age, fibrosis staging and non-SVR to IFN therapy were significant risk factors related to the development of HCC. These results were

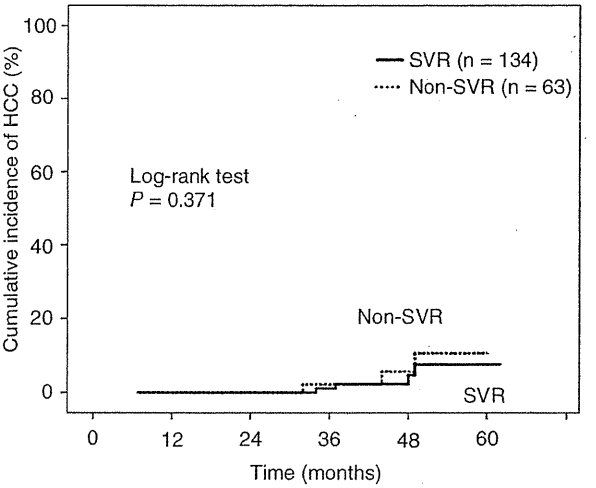

Figure 4 Cumulative incidence of hepatocellular carcinoma (HCC) development according to the treatment effect in patients who showed less than 40 IU/L average alanine aminotransferase (ALT) levels after the combination therapy. (—) Sustained virological response; (.....) non-sustained virological response.

almost comparable with those of previous reports using IFN monotherapy[12–14,21] and IFN plus ribavirin combination therapy,[22–24] suggesting that the factors associated with the development of HCC are common among these treatments and that patients of older age, with advanced fibrosis and showing non-SVR to IFN therapy should be followed up carefully for longer periods, even if IFN therapy could be performed completely. In addition, four of the SVR group patients developed HCC at more than 6 months after the treatment, which means these patients need careful follow-up even if SVR has been achieved.[25]

The incidence of HCC has been reported to be lower in patients with normal ALT levels, even if serum HCV-RNA was positive 6 or 12 months after IFN monotherapy, when compared to those without a biochemical response,[13,26,27] suggesting that the aim of IFN therapy for patients infected with HCV should be not only HCV eradication, but also the achievement of a biochemical response in order to reduce the incidence of HCC. In this study, we divided the patients into two groups, one with persistently normal serum ALT levels and the other with elevated serum ALT levels based on "the average serum ALT levels" after completion of IFN therapy. We then evaluated the cumulative HCC incidence of each group using the Kaplan–Meier estimation. Our data showed that patients with continuous normalization of ALT levels have a lower possibility of HCC development than those showing elevated ALT after the combination therapy, suggesting that continuous normalization of ALT levels after the combination therapy is an important factor for reducing HCC development. Interestingly, based on the Kaplan–Meier estimates of the cumulative HCC incidence according to the treatment effect in patients who showed less than 40 IU/L average ALT levels after the combination therapy, we found no difference in HCC incidence rates between the SVR group and non-SVR group. Figure 1 shows that the combination therapy is strongly associated with a reduced incidence of HCC in the patients who attain SVR, which seems to be a means for achieving normalization of serum ALT levels in HCV patients. However, it was also shown that, even in the non-SVR group, patients with persistently normal serum ALT levels achieved a reduced risk of HCC development. Taken together, our aim of treatment for patients infected with HCV is to primarily completely eradicate HCV. Next, for the non-SVR group patients, we would speculate that maintaining normalization of ALT levels by some other treatments may prevent HCC development in HCV-infected patients with abnormal serum ALT levels even if SVR is not achieved. Other treatments should be used to decrease serum ALT levels to below the upper limit of the normal range. Hopefully, the new treatments such as those with protease inhibitors can be helpful for these patients.[28]

Although IFN monotherapy in CHC patients has been demonstrated to be associated with reducing the incidence of HCC, especially in patients who attain SVR,[12–14] what actually occurs in IFN plus ribavirin combination therapy has not been clarified and the indicator for reducing HCC in patients infected with HCV has not been defined. We showed that this combination therapy could reduce the incidence of HCC and that older age, severe fibrosis and non-SVR were risk factors for HCC development. This therapy can increase the SVR patient ratio, and SVR or continuous normalization of ALT levels after combination therapy using IFN-α-2b plus ribavirin reduce the incidence of HCC in patients with HCV infection. Therefore, this therapy can not only avert the advance of the disease toward liver cirrhosis, but also decrease the risk of HCC. IFN plus ribavirin combination therapy is beneficial for HCV patients from both aspects. In conclusion, the present study shows that the attainment of SVR or continuous normalization of serum ALT levels induced by the combination therapy has a significantly beneficial effect on the clinical course of HCV patients by decreasing the incidence of HCC.

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Pegylated interferon alpha-2b (Peg-IFN α-2b) affects early virologic response dose-dependently in patients with chronic hepatitis C genotype 1 during treatment with Peg-IFN α-2b plus ribavirin

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SUMMARY. Chronic hepatitis C (CH-C) genotype 1 patients who achieved early virologic response have a high probability of sustained virologic response (SVR) following pegylated interferon (Peg-IFN) plus ribavirin therapy. This study was conducted to evaluate how reducing drug doses affects complete early virologic response (c-EVR) defined as hepatitis C virus (HCV) RNA negativity at week 12. Nine hundred eighty-four patients with CH-C genotype 1 were enrolled. Drug doses were evaluated independently on a body weight base from doses actually taken. From multivariate analysis, the mean dose of Peg-IFN α-2b during the first 12 weeks was the independent factor for c-EVR ($P = 0.02$), not ribavirin. The c-EVR rate was 55% in patients receiving ≥1.2 μg/kg/week of Peg-IFN, and declined to 38% at 0.9–1.2 μg/kg/week, and 22% in patients given <0.9 μg/kg/week ($P < 0.0001$). Even with stratified analysis according to ribavirin dose, the dose-dependent effect of Peg-IFN on c-EVR was observed, and similar c-EVR rates were obtained if the dose categories of Peg-IFN were the same. Furthermore, the mean dose of Peg-IFN during the first 12 weeks affected HCV RNA negativity at week 24 ($P < 0.0001$) and SVR ($P < 0.0001$) in a dose-dependent manner. Our results suggest that Peg-IFN was dose-dependently correlated with c-EVR, independently of ribavirin dose. Thus, maintaining the Peg-IFN dose as high as possible during the first 12 weeks can yield HCV RNA negativity and higher c-EVR rates, leading to better SVR rates in patients with CH-C genotype 1.

Keywords: chronic hepatitis C, drug dose, early virologic response, HCV RNA negativity, pegylated interferon plus ribavirin, sustained virologic response.

INTRODUCTION

Pegylated interferon (Peg-IFN) plus ribavirin therapy can improve anti-viral efficacy for patients with chronic hepatitis C [1–5], and the prognosis of patients in whom hepatitis C virus (HCV) is successfully eradicated improves markedly [6–10]. However, HCV still persists in approximately half of genotype 1 patients treated with Peg-IFN plus ribavirin [2–4]. Therefore, the treatment method needs to be well managed in order to maximize the virologic response in these patients with HCV genotype 1.

Abbreviations: c-EVR, complete EVR; CH-C, chronic hepatitis C; EVR, early virologic response; G-CSF, granulocyte-macrophage colony stimulating factor; Hb, haemoglobin; HCV, hepatitis C virus; Peg-IFN, pegylated interferon; Plt, platelet; SVR, sustained virologic response; WBC, white blood cell.

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In order to achieve sustained virologic response (SVR), earlier virologic response is very important for patients with chronic hepatitis C (CH-C) genotype 1. A high SVR rate (65–72%) was found in patients who achieved early virologic response (EVR) defined as a 2-log decrease in HCV RNA level at week 12, but only 0–3% SVR was seen in patients without EVR [3,11]. Additionally, complete EVR (c-EVR), which means HCV RNA negativity at week 12, is more strongly related to SVR [3].

The relationship between drug exposure and anti-viral effect has been reported in several papers [2,11–15]. McHutchison *et al.* [12] demonstrated that the SVR rate in patients who received ≥80% of their total planned doses of Peg-IFN and ribavirin for ≥80% of the scheduled duration of therapy was significantly higher than that of patients who received <80% of one or both drugs (51% *vs* 34%) and also suggested that the impact of dose reduction was greatest in patients for whom the dose had to be decreased within the first 12 weeks of treatment. In a subsequent analysis, reducing the dose of Peg-IFN and ribavirin to <80% of the full planned dose within the first 12 weeks was reported to reduce EVR rate from 80 to 33% [11]. Thus, drug adherence during the first 12 weeks has been shown to be very important for attaining EVR and SVR, but it remains obscure whether either drug can be reduced to a certain degree without adversely affecting the treatment efficacy.

In the present study, we examined the correlation between c-EVR and drug doses which are evaluated on a body weight basis from drug doses actually taken, in order to clarify the necessary drug exposure of Peg-IFN and ribavirin for achieving a higher c-EVR rate in patients with CH-C genotype 1.

PATIENTS AND METHODS

Patients

The current study was a retrospective, multicenter trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. A total of 984 patients with CH-C treated with a combination of Peg-IFN α-2b plus ribavirin were enrolled in this study between December 2004 and September 2006. The baseline characteristics of the patients are summarized in Table 1. All patients were Japanese, their mean age was 56.3 ± 10.1 years, and 56% were males. The mean serum alanine aminotransferase level was 79 ± 61 IU/L.

Patients eligible for this study were those who were infected with HCV genotype 1 and had a viral load of more than 10^5 IU/mL, but were negative for hepatitis B surface antigen or anti-human immunodeficiency virus. Patients were excluded from this study if they had decompensated cirrhosis or other forms of liver disease (alcohol liver disease, autoimmune hepatitis). Informed consent was obtained from each patient included in this study. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from each patient.

Table 1 Baseline characteristics of patients

Factor	Mean ± SD or number
n	984
Age (year)	56.3 ± 10.1
Sex: male/female	555/429
Body weight (kg)	61.8 ± 11.5
History of interferon treatment	
Naïve/experienced (relapser/nonresponder)*	575/409(160/182)
White blood cells (per mm^3)	5052 ± 1550
Neutrophils (per mm^3)	2577 ± 1092
Red blood cells ($\times 10^4/mm^3$)	442 ± 47
Haemoglobin (g/dL)	14.1 ± 1.4
Platelets ($\times 10^4/mm^3$)	15.9 ± 5.5
AST (IU/L)	66 ± 45
ALT (IU/L)	79 ± 61
Serum HCV RNA (kIU/mL)†	1600
Histology (METAVIR)‡	
Fibrosis; 0/1/2/3/4	49/314/197/105/18
Activity; 0/1/2/3	23/329/304/27

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.
*Viral response to previous treatment was unknown in 57 patients, and 10 patients had discontinued treatment. †Data shown are median values. ‡301 missing.

Treatment

All patients received Peg-IFN α-2b (PEGINTRON; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (REBETOL; Schering-Plough) for the duration of the study of 48 weeks. Peg-IFN α-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 haematological adverse effects. The dose of Peg-IFN α-2b was reduced to 50% of the assigned dose if the white blood cell (WBC) count declined to <1500/mm^3, the neutrophil count to <750/mm^3 or the platelet (Plt) count to <8 × $10^4/mm^3$, and was discontinued if the WBC count declined to <1000/

mm^3, the neutrophil count to $<500/mm^3$ or the Plt count to $<5 \times 10^4/mm^3$. Ribavirin was also reduced from 1000 to 600 mg, or 800 to 600 mg, or 600 to 400 mg if the haemoglobin (Hb) level decreased to <10 g/dL, and was discontinued if the Hb level decreased to <8.5 g/dL. Both Peg-IFN α-2b and ribavirin had to be discontinued if there was a need to discontinue one of the drugs. During this therapy, ferric medicine or haematopoetic growth factors, such as erythropoietin alpha, or granulocyte-macrophage colony stimulating factor (G-CSF), were not administered.

Virologic assessment and definition of virologic response

Serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 kIU/mL; Roche Diagnotics, Branchburg, NJ, USA) and qualitatively analysed using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/mL). The c-EVR was defined as the absence of detectable serum HCV RNA at treatment week 12, and SVR was defined as the absence of detectable serum HCV RNA at week 72. Patients with less than a 2-log decrease in HCV RNA level at treatment week 12 compared with the baseline had to stop treatment and were regarded as nonresponders. All patients with detectable serum HCV RNA at treatment week 24 were also considered nonresponders and excluded from further treatment.

Assessment of drug exposure

The amounts of Peg-IFN α-2b and ribavirin actually taken by each patient during the first 12 weeks of the treatment were evaluated by reviewing the medical records. The mean doses of both drugs were calculated individually as averages on the basis of body weight at baseline: Peg-IFN α-2b expressed as μg/kg/week, and ribavirin expressed as mg/kg/day.

Evaluation of impact of drug exposure on c-EVR

We evaluated the relationship between the drug exposure of both drugs and c-EVR by univariate and multivariate analysis for c-EVR, using the factors of mean administration doses of both drugs during the first 12 weeks and the factors at baseline. Furthermore, Peg-IFN α-2b dose (average dose per body weight and per week) was classified into five categories (up to 0.6 μg/kg; from 0.6 to <0.9 μg/kg; from 0.9 to <1.2 μg/kg; from 1.2 to <1.5 μg/kg; from 1.5 μg/kg and above). Ribavirin exposure was classified into four categories (up to 8 mg/kg; from 8 to <10 mg/kg; from 10 to <12 mg/kg; from 12 mg/kg and above), in order to examine the impact of Peg-IFN dose exposure on c-EVR. This impact was also evaluated based on the percentage of the total prescribed dose and compared with that based on the mean dose per body weight.

Statistical analysis

Baseline data for various demographic, biochemical and virologic characteristics of the patients are expressed as mean ± SD or median values. To analyse the relationship between baseline data including drug exposure and c-EVR, univariate analysis using the Mann–Whitney *U*-test or chi-squared test and multivariate analysis using logistic regression analysis were performed. The significance of trends in values was determined with the Mantel–Haenszel chi-square test. A two-tailed *P*-value < 0.05 was considered significant. Statistical analysis was conducted with SPSS version 15.0J (SPSS Inc., Chicago, IL, USA).

RESULTS

Progress of patients treated with Peg-IFN α-2b and ribavirin

Of the 984 patients, 81 discontinued treatment because of adverse events (n = 74) or voluntary withdrawal (n = 7) by treatment week 12. The 903 patients who completed 12 weeks of treatment were assessed for c-EVR. During 12–48 weeks of treatment, 331 of the nonresponders and nine of breakthrough discontinued treatment, as did 91 patients (adverse events, n = 71; voluntary withdrawal, n = 20). A total of 472 patients completed 48 weeks of treatment.

Drug reduction and virologic response

Peg-IFN α-2b was reduced without discontinuation in 29% (n = 266) and ribavirin was reduced without discontinuation in 40% (n = 359) of the 903 patients who completed 12 weeks of treatment. The c-EVR rate was 49% (445/903) and HCV RNA was negative at week 24 in 60% (542/903) of patients who completed 12 weeks of treatment. Of the 445 patients with c-EVR, 327 patients achieved SVR (73%). Only 7% of the 458 patients without c-EVR did so.

Impact of dose exposure of Peg-IFN α-2b and ribavirin on c-EVR

The mean dose of Peg-IFN α-2b actually taken during the first 12 weeks by each patient was 1.33 μg/kg/week (range 0.41–2.16 μg/kg/week; median 1.40 μg/kg/week) and that of ribavirin was 10.4 mg/kg/day (range 2.9–16.2 mg/kg/day; median 10.6 mg/kg/day).

The mean doses of both drugs and the factors at baseline correlated with the c-EVR were assessed by univariate and multivariate logistic regression analyses. Univariate analysis showed that factors significantly associated with c-EVR were age, sex, WBC, neutrophils, red blood cells, Hb, Plt, aspartate aminotransferase, the degree of liver fibrosis and the mean doses of Peg-IFN α-2b and ribavirin during the first 12 weeks (Table 2). The factors selected as significant by the univari-

Table 2 Univariate analysis for c-EVR among patients who completed 12 weeks treatment

Factor	c-EVR (+)	c-EVR (−)	*P*-value
n	445	458	
Age (year)	54.4 ± 10.4	57.5 ± 9.6	<0.001
Sex: male/female	267/178	237/221	0.01
Serum HCV RNA (kIU/mL)*	1500	1600	0.28
White blood cells (per mm^3)	5336 ± 1536	4818 ± 1547	<0.001
Neutrophils (per mm^3)	2789 ± 1133	2398 ± 1038	<0.001
Red blood cells ($\times 10^4/mm^3$)	450 ± 46	435 ± 49	<0.001
Haemoglobin (g/dL)	14.3 ± 1.4	13.9 ± 1.4	<0.001
Platelets ($\times 10^4/mm^3$)	17.3 ± 5.2	15.0 ± 5.6	<0.001
AST (IU/L)	62 ± 44	69 ± 44	<0.001
ALT (IU/L)	77 ± 64	80 ± 57	0.07
Histology (METAVIR)†			
Fibrosis: 0–2/3–4	273/37	247/74	<0.001
Activity: 0–1/2–3	171/139	159/162	0.16
Peg-IFN dose (μg/kg/week)‡	1.39 ± 0.22	1.28 ± 0.30	<0.001
Ribavirin dose (mg/kg/day)‡	10.6 ± 1.7	10.1 ± 2.1	0.002

c-EVR, complete early virologic response; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Peg-IFN, pegylated interferon.
*Data shown are median values. †272 missing. ‡Mean doses during 0–12 weeks.

Table 3 Multivariate analysis for c-EVR among patients who completed 12 weeks treatment

Factor	Category	Odds ratio	95% CI	*P*-value
Age	by 1 year	0.982	0.966–0.999	0.04
Sex	male/female	–	–	NS
Neutrophils	by 100/mm^3	1.017	1.002–1.033	0.03
Red blood cells	by 1 × $10^4/mm^3$	–	–	NS
Haemoglobin	by 1 g/dL	–	–	NS
Platelets	by 1 × $10^4/mm^3$	1.051	1.014–1.088	<0.01
AST	by 1 IU/L	–	–	NS
Fibrosis*	0–2/3–4	–	–	NS
Peg-IFN dose†	by 0.1 μg/kg/week	1.079	1.011–1.151	0.02
Ribavirin dose†	by 1 mg/kg/day	–	–	NS

95% CI, 95% confidence interval; Peg-IFN, c-EVR, complete early virologic response; pegylated interferon; N.S., No Significant difference; AST, aspartate aminotransferase.
*METAVIR fibrosis score. †Mean doses during 0–12 weeks.

ate analysis were evaluated by multivariate logistic regression analysis. The mean dose of Peg-IFN α-2b during the first 12 weeks was the independent factor for c-EVR ($P = 0.02$), apart from the neutrophils ($P = 0.03$) and Plt value at baseline ($P < 0.01$) and age ($P = 0.04$) (Table 3). In contrast, the mean dose of ribavirin during the first 12 weeks showed no correlation with c-EVR.

The c-EVR rates were 54% (137/253) and 56% (246/443) for patients who received ≥1.5 and 1.2–1.5 μg/kg/week of Peg-IFN α-2b on average during the first 12 weeks, and declined to an average rate of 38% (40/105) in patients given 0.9–1.2 μg/kg/week of Peg-IFN α-2b, and an average rate of 22% (22/102) in patients given <0.9 μg/kg/week ($P < 0.0001$) (Table 4). The c-EVR rate among the patients with ≥1.2 μg/kg/week of Peg-IFN α-2b was significantly higher than that of the patients with <1.2 μg/kg/week [≥1.2 μg/kg/week, 55% (383/696) *vs* <1.2 μg/kg/week, 30% (62/207), $P < 0.0001$].

Next, we analysed the impact of Peg-IFN α-2b on c-EVR in stratified analysis according to ribavirin dose. Figure 1 shows the relationship of c-EVR and the degree of Peg-IFN α-2b exposure for two groups of ribavirin doses: the group with ≥10.6 mg/kg/day of ribavirin and that with <10.6 mg/kg/day (10.6 mg/kg/day was the median value). In either group, the mean dose of Peg-IFN α-2b was dose-dependently correlated with c-EVR ($P < 0.0001$), and c-EVR rates were very similar in both groups if the dose categories of Peg-IFN α-2b were the same.

Table 4 The c-EVR rate according to Peg-IFN and ribavirin doses during weeks 0–12 for patients who completed 12 weeks treatment

Ribavirin dose (mg/kg/day)**	Peg-IFN α-2b dose (μg/kg/week),*				
	≥1.5	1.2–1.5	0.9–1.2	<0.9	Total
≥12	57% (60/105)	61% (22/36)	38% (6/16)	22% (2/9)	54% (90/166)
10–12	54% (46/85)	58% (154/267)	36% (14/39)	23% (11/47)	51% (225/438)
8–10	50% (25/50)	53% (52/99)	52% (15/29)	18% (4/22)	48% (96/200)
<8	46% (6/13)	44% (18/41)	24% (5/21)	21% (5/24)	34% (34/99)
Total	54% (137/253)	56% (246/443)	38% (40/105)	22% (22/102)	49% (445/903)

c-EVR, complete early virologic response; Peg-IFN, pegylated interferon.
*$P < 0.0001$ for comparison of the four Peg-IFN groups. **$P = 0.05$ for comparison of the four ribavirin groups.

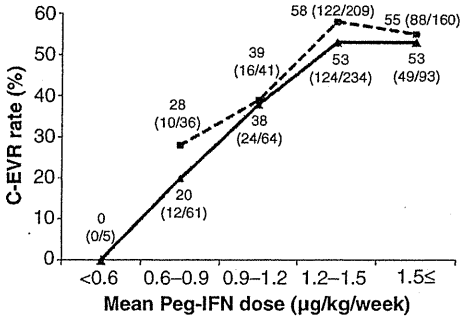

Fig. 1 Complete-EVR rate according to pegylated interferon alpha-2b (Peg-IFN α-2b) and ribavirin doses during weeks 0–12 for patients who completed 12 weeks of treatment. (-▲-) Group with the mean ribavirin dose <10.6 mg/kg/day. (--■--) Group with the mean ribavirin dose ≥10.6 mg/kg/day. The Peg-IFN α-2b dose was dose-dependently correlated with c-EVR in both groups ($P < 0.0001$). There was no significant difference between the two ribavirin-dose groups ($P = 0.19$).

c-EVR rates according to Peg-IFN α-2b drug exposure using a percentage cut off and mean dose cut off

Table 5 shows the c-EVR rates according to the category of Peg-IFN α-2b doses during the first 12 weeks based on the percentage of the total prescribed dose and the mean doses. The whole c-EVR rate was 54% (377/698) for patients who received more than 80% of the prescribed dose, and 43% (47/109) in patients given 60–80% of the prescribed dose, and 21% (21/96) in patients given <60% of the prescribed dose of Peg-IFN α-2b. Among patients given ≥80% of the prescribed dose of Peg-IFN α-2b, the c-EVR rate was significantly lower in patients given <1.2 μg/kg/week of Peg-IFN α-2b than those given ≥1.2 μg/kg/week (32% *vs* 55%, $P < 0.05$). On the other hand, even in patients given 60–80% of the prescribed dose of Peg-IFN α-2b, if they were given ≥1.2 μg/kg/week of Peg-IFN α-2b, a higher c-EVR rate was attained in comparison with those given <1.2 μg/kg/week (71% *vs* 38%, $P = 0.01$); the c-EVR rate in patients given 60–80% of the prescribed dose and ≥1.2 μg/kg/week of Peg-IFN α-2b was not inferior to that in patients given ≥80% of the prescribed dose and ≥1.2 μg/kg/week of Peg-IFN α-2b.

Impact of dose exposure of Peg-IFN α-2b during the first 12 weeks of the treatment on HCV RNA negativity at week 24 and SVR

Patients positive for HCV RNA at week 24 week during Peg-IFN α-2b and ribavirin treatment were regarded as non-responders and stopped treatment [11]. We analysed the

Table 5 The c-EVR rate according to Peg-IFN dose during weeks 0–12 based on the percentage of the planned dose and the mean doses

Peg-IFN α-2b dose (μg/kg/week)	≥80%	60–80%	<60%	Total
≥1.2	55%* (371/679)	71%** (12/17)	–	55% (383/696)
<1.2	32% (6/19)	38% (35/92)	22% (21/96)	30% (62/207)
Total	54% (377/698)	43% (47/109)	21% (21/96)	49% (445/903)

c-EVR, complete early virologic response; Peg-IFN, pegylated interferon.
*$P < 0.05$; patients with ≥1.2 μg/kg/week *vs* <1.2 μg/kg/week among the patients with more than 80% of the total prescribed dose of Peg-IFN α-2b. **$P = 0.01$; patients with ≥1.2 μg/kg/week *vs* <1.2 μg/kg/week among the patients with more than 60–80% of the total prescribed dose of Peg-IFN α-2b.

relationship between the dose exposure to Peg-IFN α-2b during the first 12 weeks and HCV RNA negative rates at week 24 or SVR in 903 patients completing 12 weeks of treatment. As a result, HCV RNA negative rates at week 24 and SVR rates declined according to the decrease in the dose of Peg-IFN α-2b during the 12 weeks of treatment; patients given ≥1.5, 1.2–1.5, 0.9–1.2 and <0.9 μg/kg/week of Peg-IFN α-2b during the first 12 weeks of the treatment showed HCV RNA negativity of 63%, 66%, 48% and 39%, respectively ($P < 0.0001$), and SVR of 46%, 43%, 30% and 20%, respectively ($P < 0.0001$).

DISCUSSION

Adherence to ribavirin was reported to be the important factor for EVR as well as that to Peg-IFN in most previous studies [2,11,12]. However, the drug exposure of Peg-IFN α-2b and ribavirin had not been analysed independently with respect to their individual influence on the anti-viral effect in these studies. Adherence to both drugs may be related factors, i.e. most patients who can tolerate a high dose of Peg-IFN are in good condition and thus can also receive a high dose of ribavirin. In the present study, the impact of the dose of Peg-IFN α-2b and ribavirin on the anti-viral effect was evaluated by multivariate logistic regression analysis, using the mean administration doses of both drugs during the first 12 weeks and baseline factors. As a result, the dose exposure of Peg-IFN α-2b was found to be the significant factor affecting c-EVR as well as baseline factors such as age, neutrophils and Plt values, but not ribavirin. This suggests that the c-EVR rate can be raised by maintaining the dose of Peg-IFN α-2b during the first 12 weeks in patients with disadvantageous factors at baseline. In fact, the c-EVR rate was higher in those who received ≥1.2 μg/kg of Peg-IFN α-2b than in those given <1.2 μg/kg of Peg-IFN α-2b for aged patients over 60 years of age (≥1.2 μg/kg; 46% *vs* <1.2 μg/kg; 28%, $P < 0.01$) or for patients with a low Plt value ($<12 \times 10^4/mm^3$)(≥1.2 μg/kg; 45% *vs* <1.2 μg/kg; 22%, $P < 0.001$). Therefore, a marked dose reduction of Peg-IFN α-2b should not be risked at the start even for aged patients or patients with lower Plt value, which is indicative of advanced fibrosis. The administration of ≥1.2 μg/kg/week of Peg-IFN α-2b is desirable as a starting dose for achieving c-EVR even in these patients: that of <1.2 μg/kg/week can lead to a non-viral response or a late viral response. Independent evaluation of the c-EVR rate according to the degree of the ribavirin dose showed a stepwise decline as the total cumulative dose of Peg-IFN α-2b decreased. Therefore, the dose of Peg-IFN α-2b should be maintained as high as possible even in patients who have to reduce Peg-IFN α-2b to <1.2 μg/kg/week. Using G-CSF for patients who develop severe neutropenia and are forced to decrease Peg-IFN can be beneficial, especially in the first 12 weeks.

The goal of 80% of the planned drug dosage for 80% of the assigned duration was derived from an adherence criterion that had been adopted previously for assessment of the efficacy of other pharmaceutical agents, such as drugs to treat cancer and human immunodeficiency virus [16]. However, in Peg-IFN plus ribavirin therapy for patients with CH-C, the planned administration dose [17,18] differs on a body weight basis by 27% for Peg-IFN α-2b and 40% for ribavirin among patients of 50–100 kg of body weight, which would be equivalent to the same rate differences for 80% of the planned drug dosage. In detail, the target dose of Peg-IFN α-2b scheduled to be administered is 1.5 μg/kg, but the usual dose for the individual patient is from 1.28 to 1.76 μg/kg/week based on body weight among patients weighing 50–100 kg according to the practice guidelines of the American Association for the Study of Liver Diseases and the manufacturer's drug information in the USA and Europe [17,18]. The range of ribavirin dose per kg of body weight is from 12 to 20 mg/kg/day. Therefore, in this study, the drug exposure was assessed from the average dose per kg of body weight.

In the evaluation of c-EVR rates according to Peg-IFN α-2b drug exposure using a percentage cut off and mean dose cut off in this study, the c-EVR rate of patients given <1.2 μg/kg/week of Peg-IFN α-2b was low (32%) even in those who received ≥80% of the total planned doses of Peg-IFN α-2b. If given ≥1.2 μg/kg/week of Peg-IFN α-2b, the c-EVR rate (71%) in patients who received 60–80% of the total doses was not inferior to that in patients given ≥80% of the total dose of Peg-IFN α-2b (54%). This means that patients whose starting dose of Peg-IFN α-2b is <1.5 μg/kg/week should not have their dosage reduced to 80% of the planned dose (<1.2 μg/kg/week) in order to have a higher probability of c-EVR, while those given ≥1.5 μg/kg/week of Peg-IFN α-2b at the start can have their dosage reduced to 80% (≥1.2 μg/kg/week) without lowering the c-EVR rate. Thus, the drug dose on a body weight basis itself should be examined as an index of the drug exposure in order to evaluate the anti-viral effect of both drugs accurately for patients with CH-C.

As for the impact of the drug exposure to ribavirin on c-EVR, the drug dose of ribavirin during the first 12 weeks was shown to have no relationship with the c-EVR rate, although it was precisely evaluated in this study, using doses actually taken on body weight. However, ribavirin can be more effective for decreasing the viral relapse after interferon or Peg-IFN α-2b and ribavirin combination therapy in patients with CH-C genotype 1 [2,3,19–24]. Recently, Shiffman *et al.* [15] have reported that a higher starting dose of ribavirin (1000–1600 mg/day) plus a regular dose of Peg-IFN α-2b with epoetin was associated with a lower relapse rate in treatment with CH-C genotype 1. Considering the viral relapse after treatment, it is thought that the ribavirin dose should not be reduced quickly in patients with mild side effects, even though it does not affect c-EVR. In fact, among the patients who attained c-EVR, a higher rate of viral relapse was found in the patients given <10 mg/kg/day of the mean ribavirin dose during 48 weeks in comparison

with those given ≥10 mg/kg/day of the mean ribavirin dose in this study [26.9% (49/182) *vs* 12.4% (26/209), $P < 0.001$] (data not shown). It seems possible to start ribavirin at a lower dose and increase it by degrees with monitoring of Hb level during treatment of patients with mild anaemia or ischemic heart disease, because the ribavirin dose appears to affect the viral relapse as the total dose over 48 weeks, not during the first 12 weeks.

In conclusion, our results have demonstrated that Peg-IFN α-2b is dose-dependently correlated with c-EVR and maintaining as high a drug dose of Peg-IFN α-2b as possible (≥1.2 μg/kg/week) during the first 12 weeks can yield higher c-EVR rates, leading to better treatment outcomes for patients with CH-C genotype 1.

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Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin

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SUMMARY. The impact of ribavirin exposure on virologic relapse remains controversial in combination therapy with pegylated interferon (Peg-IFN) and ribavirin for patients with chronic hepatitis C (CH-C) genotype 1. The present study was conducted to investigate this. Nine hundred and eighty-four patients with CH-C genotype 1 were enrolled. The drug exposure of each medication was calculated by averaging the dose actually taken. For the 472 patients who were HCV RNA negative at week 24 and week 48, multivariate logistic regression analysis showed that the degree of fibrosis ($P = 0.002$), the timing of HCV RNA negativiation ($P < 0.001$) and the mean doses of ribavirin ($P < 0.001$) were significantly associated with relapse, but those of Peg-IFN were not. Stepwise reduction of the ribavirin dose was associated with a stepwise increase in relapse rate from 11% to 60%. For patients with complete early virologic response (c-EVR) defined as HCV RNA negativity at week 12, only 4% relapse was found in patients given ≥12 mg/kg/day of ribavirin and ribavirin exposure affected the relapse even after treatment week 12, while Peg-IFN could be reduced to 0.6 μg/kg/week after week 12 without the increase of relapse rate. Ribavirin showed dose-dependent correlation with the relapse. Maintaining as high a ribavirin dose as possible (≥12 mg/kg/day) during the full treatment period can lead to suppression of the relapse in HCV genotype 1 patients responding to Peg-IFN alpha-2b plus ribavirin, especially in c-EVR patients.

Keywords: chronic hepatitis C, drug exposure, pegylated interferon plus ribavirin, virologic relapse.

INTRODUCTION

Combination therapy of pegylated interferon (Peg-IFN) plus ribavirin is very effective for patients with chronic hepatitis C (CH-C). However, sustained virologic response (SVR) in current therapy occurs in only 40–50% of patients with hepatitis C virus (HCV) genotype 1 [1–4]. Also, SVR is reduced in patients with genotype 1 who require reduction of either Peg-IFN or ribavirin, although dose reduction has little influence on SVR in those with genotype 2 or 3 [1–3,5,6]. Therefore, it is important to clarify the degree to which these medications can be reduced without adversely affecting SVR in patients with CH-C genotype 1.

In an early report on the relationship between drug exposure and antiviral effect in patients with CH-C genotype 1, patients who received ≥80% of their total planned cumulative doses of Peg-IFN and ribavirin for ≥80% of the scheduled duration of therapy had an SVR of 51% compared with only 34% for patients who received lesser amounts of one or both

Abbreviations: CH-C, chronic hepatitis C; c-EVR, complete early virologic response; ETR, end-of-treatment virologic response; Hb, haemoglobin; HCV, hepatitis C virus; IFN, interferon; LVR, late virologic response; Peg-IFN, pegylated interferon; PP, per protocol; Plt, platelet; RVR, rapid virologic response; SVR, sustained virologic response; VR, virologic response; WBC, white blood cell.

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medications [7]. On the other hand, Shiffman *et al.* [8] recently reported that reducing ribavirin did not affect SVR as long as the dose of Peg-IFN was maintained, while reducing the Peg-IFN dose significantly reduced SVR. The results of these observations are consistent with respect to the effect of Peg-IFN on SVR. However, what is controversial is whether or not reducing the ribavirin dose affects the antiviral effect.

Adding ribavirin to either interferon (IFN) or Peg-IFN monotherapy for patients with CH-C genotype 1 has been shown to reduce the relapse rate in large randomized trials [1,2,9–11]. In detail, adding ribavirin to the usual IFN monotherapy (3MIU, three-times-weekly) in 48-week treatment raised the end-of-treatment virologic response (ETR) rate from approximately 30% to 50% and also lowered the relapse rate from mid-40% to approximately 20% [9–11]. Lindsay *et al.* [12] reported that Peg-IFN alpha-2b (Peg-IFN α-2b) monotherapy (1.5 μg/kg, once-weekly), as compared with IFN alpha-2b (IFNα-2b) monotherapy (3MIU, three-times-weekly), improved ETR (49% vs. 24%), but not the relapse rate (53% vs. 50%). In the trial of Peg-IFN alpha-2a (Peg-IFN α-2a) plus ribavirin *vs* IFN α-2b plus ribavirin or Peg-IFN α-2a alone, the ETR rates were 69%, 52% and 59%, and the relapse rates were 19%, 15% and 52%, respectively [2]. These findings from large-scale trials indicate that the main role of ribavirin is to reduce relapse in the combination therapy with Peg-IFN, although ribavirin affects both ETR and relapse in combination therapy with the usual IFN.

In the present study, we tried to determine whether or not dose reduction of ribavirin (or Peg-IFN) has an effect on virologic relapse in Peg-IFN plus ribavirin treatment for patients with CH-C genotype 1.

PATIENTS AND METHODS

Patients

This study was a multicentre trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. A total of 984 patients with CH-C were enrolled in this study between December 2004 and September 2006, and treated with a combination of Peg-IFN α-2b plus ribavirin. The baseline characteristics of the patients are shown in Table 1. All patients were Japanese infected with HCV genotype 1 and a viral load of more than 10^5 IU/mL. Patients were excluded from this study if they had decompensated cirrhosis or other forms of liver disease (alcohol liver disease, autoimmune hepatitis), coinfection with hepatitis B or anti-human immunodeficiency virus. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from each patient.

Treatment

All patients received Peg-IFN α-2b (PEGINTRON; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (REBETOL; Schering-Plough) for the duration of the study of 48 weeks. As a starting dose, Peg-IFN α-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 the manufacturer's drug information available in Japan.

Table 1 Baseline characteristics of patients and drug doses at start of treatment

Factor	Mean ± SD or *n*
n	984
Age (years)	56.3 ± 10.1
Sex (male/female)	555/429
Body weight (kg)	61.8 ± 11.5
History of IFN treatment Naïve/experienced (relapser/nonresponder)*	575/409 (160/182)
White blood cells (/mm^3)	5052 ± 1550
Neutrophils (/mm^3)	2577 ± 1092
Red blood cells (×10^4/mm^3)	442 ± 47
Haemoglobin (g/dL)	14.1 ± 1.4
Platelets (×10^4/mm^3)	15.9 ± 5.5
AST (IU/L)	66 ± 45
ALT (IU/L)	79 ± 61
Serum HCV RNA (kIU/mL)†	1600
Histology (METAVIR)‡	
Fibrosis; 0/1/2/3/4	49/314/197/105/18
Activity; 0/1/2/3	23/329/304/27
Peg-IFN dose (μg/kg/week)	1.45 ± 0.17
Ribavirin dose (mg/kg/day)	11.4 ± 1.6

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus. *Viral response to previous treatment was unknown in 57 patients, and 10 patients had discontinued treatment. †Data shown are median values. ‡301 missing.

Dose reduction and discontinuance

Dose modification also followed, as a rule, the manufacturer's drug information according to the intensity of the haematologic adverse effects. The dose of Peg-IFN α-2b was reduced to 50% of the assigned dose when the white blood cell (WBC) count was below 1500/mm^3, the neutrophil count below 750/mm^3 or the platelet (Plt) count below 8×10^4/mm^3, and was discontinued when the WBC count was below 1000/mm^3, the neutrophil count below 500/mm^3 or the Plt count below 5×10^4/mm^3. Ribavirin was also reduced from 1000 mg to 600 mg, 800 mg to 600 mg, or 600 mg to 400 mg when the haemoglobin (Hb)