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Prediction of treatment outcome with daily high-dose IFN α-2b plus ribavirin in patients with chronic hepatitis C with genotype 1b and high HCV RNA levels: relationship of baseline viral levels and viral dynamics during and after therapy

Shiro Iino[a,*], Eiichi Tomita[b], Hiromitsu Kumada[c], Hiroshi Suzuki[d], Joji Toyota[e], Kendo Kiyosawa[f], Kyuichi Tanikawa[g], Michio Sata[h], Norio Hayashi[i], Shinichi Kakumu[j], Takashi Matsushima[k], Tomoyoshi Ohno[l]

[a] *Kiyokawa Hospital, 2-31-12, Asagaya Minami, Suginami-ku, Tokyo 166-0004, Japan*
[b] *Gifu Municipal Hospital, Gifu, Japan*
[c] *Toranomon Hospital, Tokyo, Japan*
[d] *Yamanashi University, Nakakoma, Japan*
[e] *Sapporo Kosei General Hospital, Sapporo, Japan*
[f] *Shinsyu University School of Medicine, Matsumoto, Japan*
[g] *International Institute for Liver Research, Japan*
[h] *Kurume University School of Medicine, Kurume, Japan*
[i] *Osaka University School of Medicine, Suita, Japan*
[j] *Aichi Medical University, Nagakute, Japan*
[k] *Municipal Hakodate Hospital, Hakodate, Japan*
[l] *Nagoya City University School of Medicine, Nagoya, Japan*

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Abstract

Data on 334 patients with HCV genotype 1b and high viral levels were extracted from two multicenter double-blind studies conducted in Japan comparing IFN α-2b plus ribavirin ($n = 209$) with IFN α-2b alone ($n = 125$) for 24 weeks. HCV RNA assay was conducted before and 4, 12, and 24 weeks after the start and 4, 12, and 24 weeks after the end of treatment. Both sustained viral response (SVR) rate and relapse rate after the end of treatment were analyzed in relation to baseline viral levels and the time of first disappearance of virus. In the combination treatment group, the percentage of patients who were HCV RNA-negative within 4 weeks decreased with increase in baseline viral levels (i.e. 42%, 15%, and 11% were HCV RNA-negative in the groups exhibiting <500, 500 to <850, and ≥850 kcopies/mL, respectively). In the IFN monotherapy group, the response rates were lower at 13%, 15%, and 1%, respectively. Disappearance of virus within 12 weeks after the start of combination treatment was indicative of higher probability of SVR. The risk of relapse was more highly correlated with the timing of initial viral disappearance than with baseline HCV levels; it was 4.8 and 10.3 times higher in patients who became HCV-negative at 4–12 and 13–24 weeks compared with in those who were HCV-negative within 4 weeks.

Keywords: Chronic hepatitis C; Interferon α-2b; Ribavirin; Multicenter randomized double-blind study

1. Introduction

Global consensus obtains that PEG-interferon (PEG-IFN) plus ribavirin combination therapy is the treatment of choice

* Corresponding author. Tel.: +81 3 3312 0151; fax: +81 3 3312 2222.

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for chronic hepatitis C (CHC). That the duration of treatment should be 12 months for hepatitis C virus (HCV) genotype 1 and 6 months for other genotypes is also nearing consensus [1,2]. High-dose daily IFN monotherapy in patients with CHC was originally reported from Japan [3], and since then several reports of better efficacy using high-dose daily IFN therapy similar to that used in Japan plus ribavirin have appeared in both the USA and Europe [4–12].

Recently, much attention has been focused on the relationship between the timing of HCV RNA negativity and antiviral efficacy of IFN therapy. Many reports have investigated this relationship in patients receiving IFN or PEG-IFN and ribavirin combination therapy [13–18]. In Japan, the age of patients with CHC is increasing, and both nonresponders to previous IFN therapy and IFN-treatment-naïve patients usually are given high doses of IFN. In two Japanese studies of high-dose IFN therapy, SVR including in patients with HCV with genotype other than 1 was observed in 27.5% (316/1148) [19] and 30.6% (313/1022) [20], respectively, whereas in the USA and Europe where IFN 3 MIU is normally administered three times/week, SVR was observed in 6–19% even in patients undergoing treatment for 1 year [21–23]. For this reason, Japanese nonresponders to prior IFN therapy cannot be considered the same as non-Japanese patients, and hence direct application of the results of trials conducted outside Japan to Japanese patients is of limited use.

It has also been reported that reducing HCV relapse after the end of treatment enhances the efficacy of combination therapy [24]. Longer-term combination treatment has been confirmed to reduce the rate of HCV relapse after the end of drug administration [22,23], but the mechanism of this effect is not clear. The present study was performed to examine the relationship between the timing of disappearance of HCV RNA and HCV eradication in Japanese patients receiving IFN plus ribavirin combination therapy. Moreover, we attempted to clarify factors related to relapse after the end of treatment by analyzing the relationship between the time of HCV eradication and baseline HCV levels.

2. Materials and methods

2.1. Patient selection

Two randomized comparative studies of IFN α-2b plus ribavirin were conducted using IFN (-2b monotherapy as control; 1 in patients with HCV genotype 1b CHC with high viral levels (the most difficult CHC patients to treat) [25] and 1 in nonresponders and relapsers to previous IFN therapy [26] who are thus in urgent medical need. No bias was observed in patient distribution between groups in these studies (data not shown). Both studies were conducted after approval by the institutional review boards of each medical institution and informed consent was obtained in writing from each patient. IFN α-2b (Intron A, Schering Plough, Kenilworth, NJ) was administered six times/week for 2 weeks at a dose of 6 or 10 MIU and then three times/week for 22 weeks at a dose of 6 MIU. Ribavirin (Rebetol, Schering Plough, Kenilworth, NJ) was administered for 24 weeks at a dose of 600 mg/day (three capsules) in patients weighing <60 kg and 800 mg/day (four capsules) in those whose weight was ≥60 kg. The control group received IFN (-2b together with ribavirin placebo capsules. From these two clinical studies, we extracted data on patients with HCV genotype 1 and high viral levels and retrospectively analyzed the time of initial HCV RNA negativity, the percentage of patients with sustained viral negativity after the end of treatment, and the percentage of patients who relapsed after the end of treatment. The database subjected to retrospective analysis included data on sex, age, body weight, extent and activity of liver tissue lesion, history of IFN therapy, HCV RNA level, aspartate aminotransferase (AST), alanine aminotransferase (ALT), hemoglobin, white blood cells (WBC), red blood cells (RBC), platelet count, and serum creatinine. Virological response was defined as qualitative negative by qualitative Amplicor assay (Mitsubishi Kagaku BCL, Tokyo, Japan). In addition, HCV quantitative analysis was conducted by Amplicor HCV monitor method (Mitsubishi Kagaku BCL, Tokyo, Japan) with a detection limit of 100 copies/mL.

Qualitative and quantitative analyses of HCV RNA were performed immediately before and 4, 12, and 24 weeks after the start and 4, 12, and 24 weeks after the end of treatment. Viral levels ≥100 kcopies/mL were considered high. Genotype was determined immediately before the start of treatment by RT-PCR (Mitsubishi Kagaku BCL, Tokyo, Japan). All liver tissue was evaluated by the same examiner.

2.2. Enrollment and exclusion criteria

Enrollment criteria for the two studies were: (1) abnormal ALT and HCV RNA-positive in tests conducted within 12 weeks before the start of treatment; (2) HCV genotype 1, and HCV genotype 2 nonresponders and relapsers to prior IFN therapy; (3) age 20–64 years; (4) hemoglobin ≥12 g/dL and platelet count ≥100,000 mm^{-3} within 12 weeks before the start of treatment; (5) availability to stay in hospital for 4 weeks after the start of treatment; and (6) agreement to take contraceptive measures during and for 6 months after the end of treatment. Patients with the following characteristics were excluded: (1) pregnant or possibly pregnant and lactating women; (2) depression tendency; (3) severe complications; (4) hepatitis C complicated by other types of hepatitis; (5) liver cirrhosis or cancer as diagnosed in tests conducted within 12 weeks before the start of treatment; (6) history of hepatic encephalopathy, rupture of esophageal varices, or ascites; (7) HIV coinfection; (8) taken antiviral therapy or immunotherapy within 12 weeks before the start of treatment; (9) previous ribavirin therapy; and (10) history of allergy to IFN or nucleoside analogues.

2.3. Subgroup analysis

Baseline HCV RNA levels were categorized into three groups: 100 to <500; 500 to <850; and ≥850 kcopies/mL as determined by Amplicor HCV monitor assay. Disappearance of virus and relapse were judged by qualitative Amplicor assay. The time of initial HCV RNA negativity was recorded as the measurement time point (4, 12, and 24 weeks after the start of treatment) at which negativity was first observed; the time of initial relapse was the three measurement time point (4, 12, and 24 weeks after the end of treatment) at which HCV RNA was first detected in patients who achieved HCV RNA negativity during the treatment period. Patients who remained HCV RNA-negative for 6 months after the end of treatment were considered to have achieved SVR.

2.4. Statistical analysis

HCV RNA negativity rate, SVR rate, and relapse rate were compared by baseline viral load between the combination treatment and IFN monotherapy groups by Mantel–Haenzel test using modified RIDIT scores after the lack of interactions in efficacy was confirmed by the Breslow–Day test. Logistic regression analysis was used to identify factors contributing to initial HCV RNA negativity and SVR. The degree of risk of relapse was analyzed using the proportional hazards and grouped exponential models. Intergroup differences in patient profiles were tested by Fisher's exact test, Wilcoxon–Mann Whitney test, and Mantel–Haenzel test. $P < 0.05$ was regarded as statistically significant (two-sided). All calculations were performed by SAS program version 6.12 (SAS Institute, Cary, NC).

3. Results

3.1. Patient characteristics

Table 1 shows the main characteristics of the 209 patients in the combination treatment and 125 patients in the IFN monotherapy groups. In the study in nonresponders and relapsers to previous IFN therapy [26] 41 and 40 patients were allocated to the combination treatment and IFN monotherapy groups, respectively; in the study in patients with genotype 1b and high viral titers, the numbers were 168 and 85, respectively (i.e. 2:1 randomization) [25]. A total of 107 patients (51%) in the combination treatment and 68 (54%) in the IFN monotherapy group had HCV RNA levels ≥850 kcopies/mL. About half of patients in both treatment groups were relapsers after previous IFN therapy. Forty-nine patients (23%) in the combination treatment and 24 (19%) in the IFN monotherapy group had not received prior IFN therapy. No imbalance was observed in background variables between the two groups.

Table 1
Baseline patient characteristics

	IFN + ribavirin	IFN	*P* value
No. of patients	209	125	
Sex (male/female)	164/45	94/31	0.503[a]
Mean age (years)	48	49	0.539[b]
Viral load (kcopies/mL)			0.792[c]
Low (<500)	23.0% (48)	24.8% (31)	
Moderate (500 to <850)	25.8% (54)	20.8% (26)	
High (≥850)	51.2% (107)	54.4% (68)	
Previous IFN therapy			0.295[ad]
Treatment-naive	23.4% (49)	19.2% (24)	
Relapsers	50.7% (106)	50.4% (63)	
Nonresponders	22.5% (47)	30.4% (38)	
Unknown	3.3% (7)	0	

[a] Fisher test.
[b] *U*-test.
[c] Mantel–Haenzel test.
[d] Excluding unknown.

3.2. Response to therapy

The SVR rate was 18% (38/209) with IFN and ribavirin combination therapy and 2% (2/125) with IFN monotherapy. The results of subgroup analysis by baseline viral levels are shown in Table 2. Patients receiving IFN and ribavirin combination therapy had a significantly higher chance for SVR than those receiving IFN monotherapy at any baseline viral level.

3.3. Initial viral negativity

In patients with viral titers of 500 to <850 kcopies/mL, initial viral negativity occurred in 20% by the first 4 weeks, in 46% by 12 weeks, and in 17% by 24 weeks of treatment in the combination therapy group (Fig. 1a). In the IFN monotherapy group the figures were 15%, 31%, and 12%, respectively (Fig. 1b). In patients with viral titers of ≥850 kcopies/mL, the HCV negativity rates at the same time points were 11%, 50%, and 20%, respectively, in the combination therapy group (Fig. 1a) and 1%, 28%, and 15%, respectively, in the monotherapy group (Fig. 1b). The time to initial viral negativity was slightly earlier in patients with viral titers of <500 kcopies/mL (42%, 25%, and 6% at the same time points, respectively) than in those with ≥500 kcopies/mL in the combination therapy group (Fig. 1a). Logistic regression analysis indicated that low HCV RNA levels and high ALT and creatinine levels before treatment are factors related to achieving HCV RNA negativity by week 4 of combination therapy. High baseline creatinine level was associated

Table 2
SVR rate by baseline viral load

Viral load (kcopies/mL)	IFN + ribavirin ($n = 209$)	IFN ($n = 125$)	*P* value
Low (<500)	29% (14/48)	0 (0/31)	0.001
Moderate (500 to <850)	17% (9/54)	8% (2/26)	0.001
High (≥850)	14% (15/107)	0 (0/68)	0.001

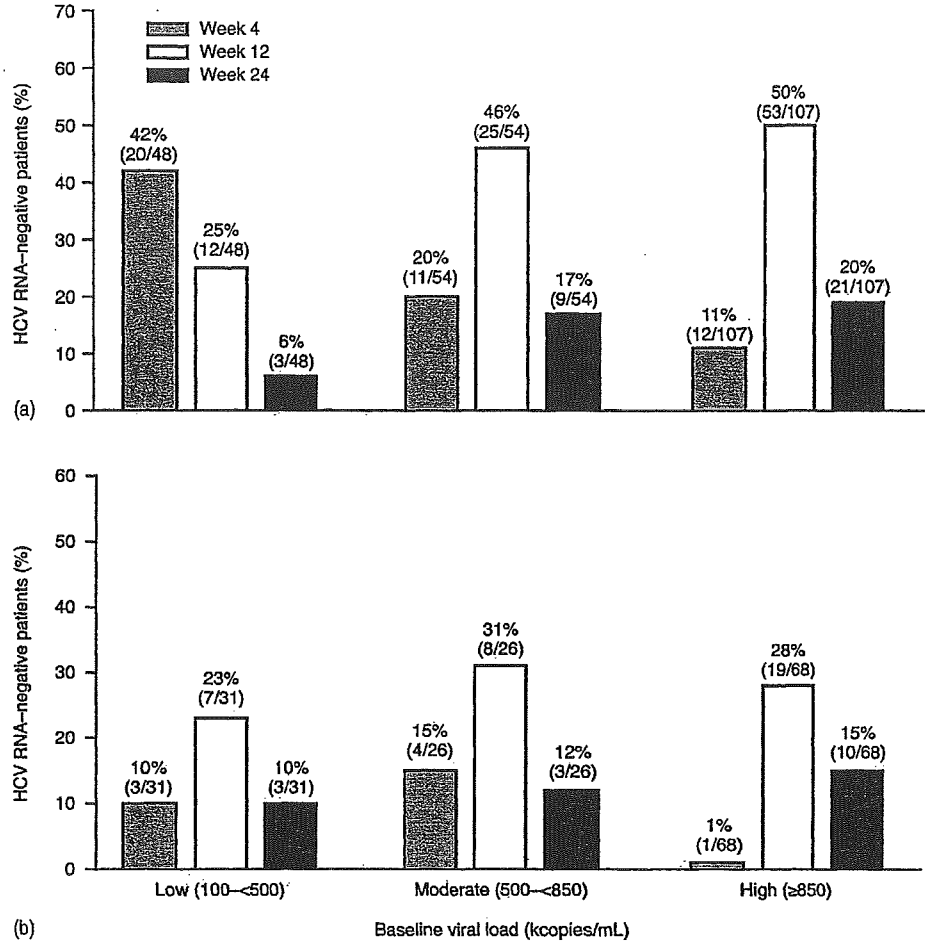

Fig. 1. Percentage of patients testing HCV RNA negative receiving combination therapy (a) and monotherapy (b). Patient numbers are in parentheses.

with achieving HCV RNA negativity by week 12 of treatment

3.4. SVR by baseline viral level and timing of initial viral negativity

Fig. 2 shows the SVR rate with respect to the timing of initial viral disappearance for each baseline HCV level. In patients with <500 kcopies/mL, SVR was observed only in those HCV RNA-negative within 4 weeks after the start of combination treatment, with a high SVR rate of 70% (14/20). However, in patients HCV RNA-negative by week 12 or 24, SVR was not observed in either treatment group. In the IFN monotherapy group, three patients were HCV RNA-negative within 4 weeks and the SVR rate was 0% (0/3). Among patients with 500 to <850 kcopies/mL, SVR was observed in 55% (6/11) and 12% (3/25) of patients HCV RNA-negative within 4 and 12 weeks of the start of combination treatment, respectively, and in none of the nine patients HCV RNA-negative within 24 weeks. Among patients treated with IFN alone, SVR was observed only in 50% (2/4) of patients HCV RNA-negative within 4 weeks. In patients with ≥850 kcopies/mL at baseline, SVR was observed in the combination treatment group in 42% (5/12) and 19% (10/53) of patients HCV RNA-negative within 4 weeks and 12 weeks, respectively. However, SVR was not seen in any of the 21 patients HCV RNA-negative within 24 weeks. SVR was not observed in patients with viral levels ≥850 kcopies/mL treated with IFN alone.

In patients HCV RNA-negative within 4 weeks, low baseline viral levels and high body weight were factors contributing to SVR; in those who were HCV RNA-negative within 12 weeks, low baseline viral levels and high baseline platelet count were contributing factors.

3.5. Relapse rate after end of treatment by baseline viral levels

In the combination treatment group, the relapse rate was 60% (21/35), 80% (36/45), and 83% (71/86) and in the IFN (-2b alone group 100% (13/13), 86% (13/15), and 100% (30/30) in patients with baseline viral levels <500, 500 to

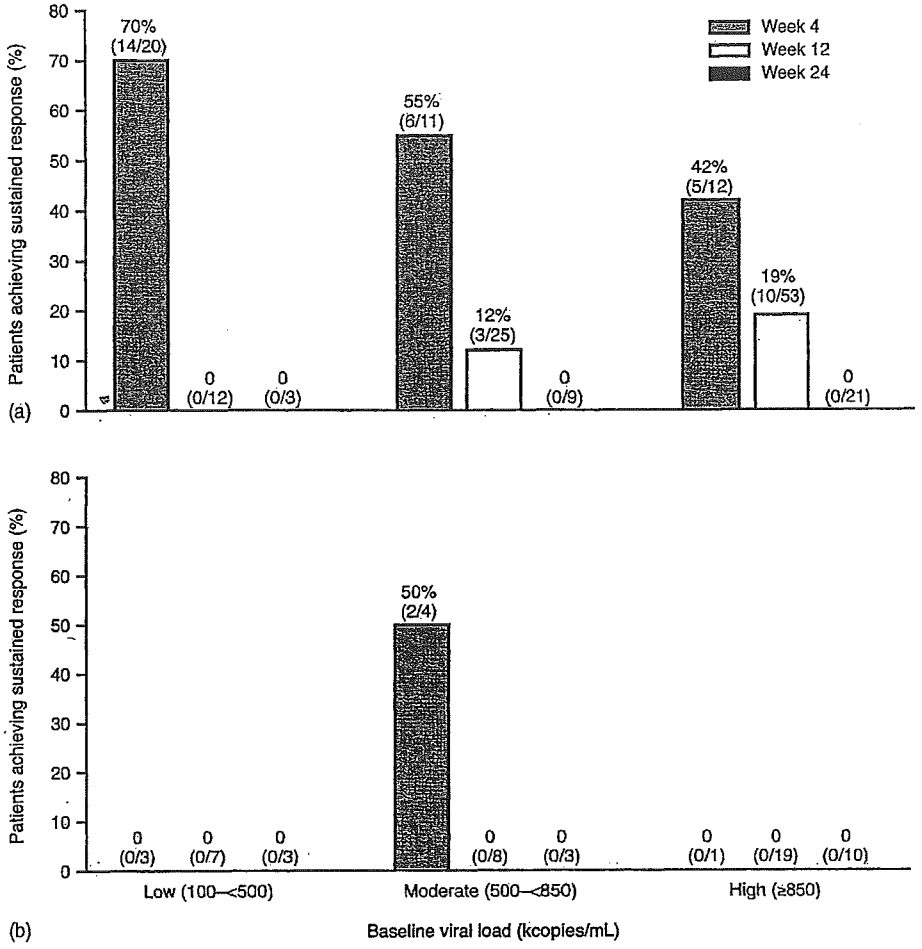

Fig. 2. Sustained response rate in patients receiving combination therapy (a) and monotherapy (b). Patient numbers are in parentheses.

<850, and ≥850 kcopies/mL, respectively (Table 3). Patients in the combination treatment group were 3.7 times (95% CI 1.9–7.3; $P < 0.001$) less likely to relapse than those in the IFN monotherapy group. If the probability of relapse in patients with <500 kcopies/mL is set at 1, then probability was 1.5 (95% CI 0.8–2.9) in patients with 500 to <850 kcopies/mL and 2.0 (95% CI 1.1–3.5) in patients with ≥850 kcopies/mL. However, baseline viral level was not a significant factor ($P = 0.3441$) with regards to risk of relapse. On the other hand, patients HCV RNA-negative within 12 and 24 weeks after the start of treatment were 4.8 (95% CI 2.8–8.1) and 10.3 (95% CI 5.4–19.7) times, respectively, more likely to relapse after the end of treatment than patients HCV RNA-negative within the first 4 weeks ($P < 0.001$).

Table 3
Rate of relapse at 6 months by baseline viral load

Viral load (kcopies/mL)	IFN + ribavirin ($n = 209$)	IFN ($n = 125$)	P value
Low (<500)	60% (21/35)	100% (13/13)	0.001
Moderate (500 to <850)	80% (36/45)	87% (13/15)	0.001
High (≥850)	83% (71/86)	100% (30/30)	0.001

The relative risk of relapse after the end of treatment by initial viral level and time of first viral negativity is shown in Table 4. The risk of relapse in the combination group versus in the IFN monotherapy group was significantly lower by a factor of 0.3 (95% CI 0.1–0.9) and 0.5 (95% CI 0.3–0.8) in patients HCV RNA-negative within 4 weeks ($P = 0.032$) and 12 weeks ($P = 0.011$), respectively. Regarding baseline HCV levels, in patients HCV RNA-negative by 12 weeks, the

Table 4
Relative risk of relapse at 6 months (95% CI)

	First HCV RNA-negative test result		
	4 weeks	12 weeks	24 weeks
Treatment group			
IFN + ribavirin	0.3 (0.1–0.9)*	0.5 (0.4–0.9)*	1.2 (0.6–2.2)
IFN	1	1	1
Baseline viral load (kcopies/mL)			
Low (<500)	1	1	1
Moderate (500 to <850)	0.9 (0.4–2.7)	0.6 (0.4–1.2)	0.8 (0.3–2.3)
High (≥850)	2.1 (0.8–5.6)	0.5 (0.3–0.9)†	0.9 (0.4–2.2)

* $P < 0.05$ vs. monotherapy.
† $P < 0.05$ vs. low baseline viral load.

hazard for relapse was significantly higher (odds ratio: 0.5; $P = 0.021$) in patients with ≥850 kcopies/mL than in those with <500 kcopies/mL. In patients HCV RNA-negative by 24 weeks, no effect on hazard for relapse was observed by treatment group or viral levels.

The relationship between baseline HCV levels and the time of relapse by time of initial HCV negativity is shown in Table 5. The relapse rate in patients HCV RNA-negative within 4 weeks with combination treatment was ≤18% at 4, 12, and 24 weeks after the end of treatment regardless of baseline viral level. In the IFN(-2b alone group, almost all patients relapsed soon after the end of treatment even when HCV RNA-negativity occurred within the first 4 weeks after the start of treatment. The circumstance of relapse in patients HCV RNA-negative within 12 weeks of the start of treatment differed from that in patients HCV RNA-negative within 4 weeks. In those receiving combination treatment, relapse was seen within 4 weeks in 11 (92%), 13 (52%), and 30 (57%) patients whose baseline viral levels were <500, 500 to <850, and ≥850 kcopies/mL, respectively. Relapse within 12 weeks was seen in 1 (8%), 5 (20%), and 13 (26%) patients, respectively. However, even with combination treatment, most patients who first became HCV RNA-negative after 12 weeks from the start of treatment relapsed within 4 weeks after the end of treatment (data not shown)

4. Discussion

In Japan, various IFN regimens for the treatment of CHC have been tried. Under the Japanese health insurance system, the duration of treatment was restricted to 6 months at the time that the present study was conducted. Standard treatment comprises high doses of IFN (6–10 MIU) administered daily in the initial stage of treatment followed by further doses at three times/week for ≤6 months with the aim of eradicating the virus [3]. In 1998, remarkable improvement in efficacy was reported when ribavirin is added to IFN α-2b [21–23], and clinical studies of IFN α-2b plus ribavirin combination therapy were initiated in Japan. Outside Japan, the standard treatment regimen with IFN α-2b was 3 MIU administered three times/week; for combination therapy, ribavirin was added to this standard regimen. The clinical studies in Japan were likewise conducted with ribavirin (600 or 800 mg/day depending on body weight) added to the standard Japanese regimen. When SVR rates by baseline viral levels were compared, combination therapy was superior to monotherapy at all viral levels. A number of reports have been published concerning the timing of first HCV RNA disappearance and its effect on the SVR rate [13–18,27]. To date, however, no study of the SVR rate analyzed in relation to HCV RNA levels has been published. The present study suggests that the timing of first disappearance of HCV RNA is significantly affected by baseline HCV RNA levels. In patients with low baseline viral levels (100 to <500 kcopies/mL), 42% became HCV RNA-negative in comparison with only 11% with high viral levels (≥850 kcopies/mL) following 4 weeks' combination therapy. The study suggests that >4 weeks' treatment is required to achieve HCV RNA negativity in patients with viral levels >500 kcopies/mL. Moreover, with IFN alone the proportion of patients achieving HCV RNA negativity within 4 weeks was especially low among those with HCV genotype 1b and high viral levels; >4 weeks' treatment is required to achieve HCV RNA negativity in this group. Vrolijk et al. [5] reported that when ribavirin was administered in combination with IFNα-2b, HCV RNA negativity was observed by week 4 in nearly half of patients and all patients achieved SVR when treatment was continued for 1.5 years. Tassopoulos et al. [8] reported that when ribavirin was administered in combination with 10 MIU IFNα-2b for 8 weeks, almost half of patients achieved HCV RNA negativity. Treatment was continued thereafter for 48 weeks, and the final SVR rate was roughly 25%. The differences between these studies conducted outside Japan and our results may be explained by the high viral levels in our patients. We also noted that low HCV RNA, high ALT, and high creatinine levels before the start of dosing were factors associated with early disappearance of HCV RNA after treatment was initiated. High serum creatinine levels are related to high serum ribavirin concentrations [28], and this may explain early HCV RNA disappearance.

Kasahara et al. [29] compared the results of 6-month and 1-year treatment and reported that judging from the degree of improvement in ALT, longer duration of treatment with IFN monotherapy may inhibit relapse after the end of treatment. However, no significant difference of SVR rate in CHC patients with genotype 1b and high viral levels between 52 weeks and 78 weeks treatment with IFN monotherapy was reported [30]. Poynard et al. [22] reported that the relapse rate with combination therapy after 48 weeks of treatment in patients not previously treated with IFN and including patients with HCV genotypes other than genotype 1 was significantly lower than after 24 weeks of treatment. McHutchison et al. [23] also reported a similar trend. Portal et al. [6] compared the relapse rate in HCV genotype 1 patients with high viral levels treated with IFN plus ribavirin for 1 year or IFN plus ribavirin for 6 months followed by IFN monotherapy for 6 months, and observed a significantly higher relapse rate in the latter group, indicating the importance of duration of combination treatment in reducing the relapse rate. Although our study of 6-month combination treatment in genotype 1 patients was not adequate to analyze the effects of duration of treatment, our analysis of HCV RNA levels in relation to relapse revealed that relapse is much more likely in patients with high rather than low baseline viral levels. Furthermore, compared with in patients who were HCV RNA negative within 4 weeks, the relative risk for relapse is significantly higher in patients HCV RNA-negative at both 4–12 weeks and 13–24 weeks after the start of treatment. Relative risk of relapse is also reduced by about 0.5 with combination therapy compared with monotherapy. Moderate antiviral effects of ribavirin remaining in the body for long periods after end of

Table 5
Relapse rate by baseline HCV level in patients HCV RNA-negative within 4 weeks and within 12 weeks

	Baseline viral load in patients receiving combination therapy (kcopies/mL)			Baseline viral load in patients receiving monotherapy (kcopies/mL)		
	Low (<500)	Moderate (500 to <850)	High (≥850)	Low (<500)	Moderate (500 to <850)	High (≥850)
(a) HCV RNA-negative within 4 weeks						
Relapse						
4 weeks	15% (3/20)	18% (2/11)	17% (2/12)	67% (2/3)	50% (2/4)	100% (1/1)
12 weeks	5% (1/20)	9% (1/11)	17% (2/12)	0 (0/3)	0 (0/4)	0 (0/1)
24 weeks	10% (2/20)	18% (2/11)	17% (2/12)	0 (0/3)	0 (0/4)	0 (0/1)
Unknown	–	–	8% (1/12)	33% (1/3)	–	–
Total	30% (6/20)	45% (5/11)	55% (7/12)	100% (3/3)	50% (2/4)	100% (0/1)
(b) HCV RNA-negative within 12 weeks						
Relapse						
4 weeks	92% (11/12)[a]	52% (13/25)	57% (30/52)	100% (7/7)	100% (8/8)	84% (16/19)
12 weeks	8% (1/12)	20% (5/25)	26% (13/53)	0 (0/7)	0 (0/8)	5% (1/19)
24 weeks	0 (0/12)	4% (1/25)	0 (0/53)	0 (0/7)	0 (0/8)	5% (1/19)
Unknown	–	12% (3/25)	–	–	–	5% (1/19)
Total	100% (12/12)	88% (22/25)	81% (43/53)	100% (7/7)	100% (8/8)	100% (19/19)

Intergroup within 4 weeks: $P = 0.0319$; time $P = 0.0026$, intergroup within 12 weeks: $P = 0.0109$; time $P = 0.0001$.

treatment might explain the better end-of-treatment response with combination therapy [31]. Although HCV eradication may not be expected in patients HCV positive at 12 weeks, combination therapy should be continued so as to suppress liver inflammation and progression of liver cirrhosis. Moreover, the duration of treatment should be 12 months in patients with genotype 1 and high viral levels.

Many attempts have been made to improve the efficacy of combination therapy. Extending the dosing period from 6 months to 1 year does not affect the HCV RNA negativity rate at the end of treatment [22,23]; improved efficacy with the longer course is attributed to decreases in relapse rate after the end of treatment [32]. Thus it is necessary to conduct a prospective study to determine the optimal duration of combination treatment after HCV RNA becomes negative to improve the efficacy of IFN plus ribavirin combination therapy.

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Impact of daily high-dose IFNα-2b plus ribavirin combination therapy on reduction of ALT levels in patients with chronic hepatitis C with genotype 1 and high HCV RNA levels

Shiro Iino[a,*], Eiichi Tomita[b], Hiromitsu Kumada[c], Hiroshi Suzuki[d], Johji Toyota[e], Kendo Kiyosawa[f], Kyuichi Tanikawa[g], Michio Sata[h], Norio Hayashi[i], Shinichi Kakumu[j], Takashi Matsushima[k], Masashi Mizokami[l]

[a] *Kiyokawa Hospital, 2-31-12, Asagaya minami, Suginami-ku, Tokyo 166-0004, Japan*
[b] *Gifu Municipal Hospital, Gifu, Japan*
[c] *Toranomon Hospital, Tokyo, Japan*
[d] *Yamanashi University, Nakakoma, Japan*
[e] *Sapporo Kosei Hospital, Sapporo, Japan*
[f] *Shinshu University School of Medicine, Matsumoto, Japan*
[g] *International Institute for Liver Research, Kurume, Japan*
[h] *Kurume University School of Medicine, Kurume, Japan*
[i] *Osaka University School of Medicine, Osaka, Japan*
[j] *Aichi University School of Medicine, Nagakute, Japan*
[k] *Municipal Hakodate Hospital, Hakodate, Japan*
[l] *Nagoya City University School of Medicine, Nagoya, Japan*

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Abstract

The possibility of delaying progression to hepatocellular carcinoma in chronic hepatitis C patients with genotype 1 and high viral titers with baseline ALT levels of ≥50 IU/L was examined by administration of IFN plus ribavirin combination therapy using ALT normalization as index and IFN monotherapy as control. The rate of sustained ALT normalization (ALT normal at 24 weeks after the end of treatment) was 28.1% with combination therapy and 10.5% with IFN monotherapy ($P=0.001$). Furthermore, the number of patients with sustained viral response (SVR) and with sustained ALT normalization in non-SVR patients was also significantly higher in the combination therapy versus monotherapy group. Mean ALT values during treatment and for 6 months after the end of treatment were significantly lower with combination therapy versus monotherapy even in virological nonresponders, as well as significantly lower during the post-treatment observation period in patients who relapsed after the end of treatment. Since increase in the rate of sustained ALT normalization and SVR were successfully achieved, inhibition of progression to hepatocellular carcinoma should be studied with long-term IFN and ribavirin combination therapy.

Keywords: Chronic hepatitis C; IFNα-2b; Ribavirin; ALT; Hepatocellular carcinoma prevention

1. Introduction

With the aging of the chronic hepatitis C patient population in Japan, a rapid increase in the incidence of hepatocellular carcinoma (HCC) is being observed [1]. Deaths due to HCC number over 30,000 per year [1], and prevention of progression to HCC is now an urgent issue. Many reports on the efficacy of interferon (IFN) in preventing progression to HCC in patients with chronic hepatitis C have been published by Japanese researchers [2–8]. At first, normalization

* Corresponding author. Tel.: +81 3 3312 0151; fax: +81 3 3312 2222.

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of serum alanine aminotransferase (ALT) levels while on IFN therapy was thought to inhibit progression to HCC [3]. However, the results of long-term follow-up studies clearly indicate that sustained viral response (SVR) and/or sustained normalization of ALT after the end of treatment are necessary for the long-term inhibition of progression to HCC [4,6–8]. Results indicating that such inhibition is possible if ALT levels are maintained long-term within about twice the upper limit of normal (80 IU/L) have also been reported [9,10].

The standard of treatment of chronic hepatitis C worldwide is pegylated-IFN (PEG-IFN) in combination with ribavirin. The addition of ribavirin has been shown radically to increase the rate of eradication of HCV [11–13], and this is thought to result in increased inhibition of progression to HCC in patients with chronic hepatitis C. The efficacy of PEG-IFN plus ribavirin in the prevention of histologic progression using fibrosis as index has already been reported although these studies did not directly examine the effect on inhibition of progression to HCC [14,15]. Based on an average follow-up period of 20 months, one-stage improvement in METAVIR score was observed in 73% of patients on 1-year administration of PEG-Intron 1.5 μg plus ribavirin. One-stage exacerbation was observed only in 8%. Ribavirin alone has almost no effect on reducing HCV levels, but is reported to normalize ALT levels during treatment [16,17], and similar effects may be expected with combination therapy. The focus worldwide is on antiviral efficacy, and there are very few reports of detailed examination of the effect of combination therapy on liver function [18].

Recently, the efficacy of combination therapy with ribavirin in the context of inhibition of progression to HCC has started to be investigated although the number of patients involved is small compared with the numbers enrolled in clinical studies of IFN in Japan. Yang et al. [19] reported that the 7-year cumulative HCC rate is 1.4% in patients receiving IFN plus ribavirin, which is much lower than the 10.2% reported in patients receiving IFN alone, although the difference was not statistically significant due to the small sample size. It has been reported elsewhere that factors contributing to inhibition of progression to HCC include absence of liver cirrhosis before the start of treatment and sustained viral response although the data include both combination therapy and monotherapy cases [20]. The above Japanese data indicate that prevention of progression to HCC can be expected with sustained normalization of ALT, although the presence of this factor does not necessarily indicate that liver histology is normal [18]. Hence we tested the hypothesis that combination therapy consisting of IFNα-2b plus ribavirin for 24 weeks in difficult-to-treat HCV genotype 1 patients leads not only to the eradication of HCV but ultimately to the prevention of histological progression with increased normalization of liver function.

2. Materials and methods

2.1. Patient selection

Two randomized comparative clinical studies of IFNα-2b plus ribavirin combination therapy versus IFNα-2b alone were initiated in Japan in 1998 in chronic hepatitis C patients; one in difficult-to-treat genotype 1 and high viral titer (> 100 kcopies/mL) patients [21] and the other in nonresponders and relapsers to previous IFN therapy [22]. From these two studies, data on patients with ALT levels ≥50 IU/L (i.e. about 1.5 times the mean upper limit of normal) at the start of treatment were extracted and the effects of treatment on ALT improvement were retrospectively analyzed. In both studies, IFN α-2b (Intron A; Schering Plough, Kenilworth, NJ) was administered at doses of 6 or 10 MIU six times per week for 2 weeks followed by 6 MIU three times per week for 22 weeks. Patients in the combination treatment groups additionally received ribavirin (Rebetol, Schering Plough, Kenilworth, NJ) at a dose of 600 mg/day (three capsules) and 800 mg/day (four capsules) in those weighing <60 kg and ≥60 kg, respectively, for 24 weeks. Patients in the control groups took ribavirin placebo capsules. In both studies, patients were randomized to either treatment.

The studies were approved by the institutional review boards of each study site and all patients provided written informed consent to participate. Inclusion criteria were as follow: (1) HCV RNA-positive and ALT abnormal in tests conducted within 12 weeks prior to the start of treatment; (2) HCV genotype 1 or if genotype 2 nonresponder or relapser to previous IFN treatment; (3) age between 20 and 64 years; (4) hemoglobin ≥ 12 g/dL and platelets ≥ 100,000 mm^{-3} in the most recent test conducted within 12 weeks prior to the start of treatment; (5) available for hospitalization for 4 weeks after the start of treatment; and (6) contraception possible both during and for 6 months after the end of treatment. Exclusion criteria were as previously reported [23]. The database for this retrospective study included information on sex, age, body weight, histological stage and activity index, IFN treatment history, HCV RNA levels, aspartate aminotransferase, ALT, hemoglobin, white blood cells (WBC), red blood cells, platelets, and creatinine.

2.2. Study design

Pretreatment ALT levels were classified into three grades: 50 to <100 IU/L, 100 to <150 IU/L, and ≥150 IU/L. The effect of timing of initial ALT normalization on sustained ALT normalization was examined as well as the association between virological efficacy and improvement in liver function. ALT was measured before and 1–4, 6, 8, 12, 16, 20, and 24 weeks after the start and 2, 4, 8, 12, 16, 20, and 24 weeks after the end of treatment. The judgment of ALT normalization and less than two times of upper normal ALT levels were made based on the normal values at each study site (median 37.5 IU/L, range 21–50 IU/L), and the timing of

initial ALT normalization was recorded as the day when the judgment of normal ALT was made for the first time during treatment. HCV RNA was measured before and 4, 12, and 24 weeks after the start and 24 weeks after the end of treatment. HCV RNA was measured by qualitative Amplicor assay (Mitsubishi Kagaku BCL, Tokyo, Japan), and genotype determined before the start of treatment by reverse transcriptase polymerase chain reaction (Mitsubishi). Evaluation of liver histology was conducted by a single evaluator based on liver tissue samples taken within 48 weeks prior to the start of treatment.

2.3. Definition of response

ALT normalization at 24 weeks after the end of treatment was considered "effective" and was the primary endpoint of this examination. Separately, the association with virological efficacy was also examined. HCV RNA negativity by qualitative assay at 24 weeks after the end of treatment was defined as sustained viral response. Virological relapsers were patients who were HCV RNA-negative by qualitative assay at the end of treatment but who became HCV RNA-positive after the end of treatment. Nonresponders were patients who were never HCV RNA-negative during or after treatment.

2.4. Statistical analysis

After confirming the absence of interaction in efficacy by the Breslow–Day test, comparison of sustained ALT normalization rate by pretreatment ALT levels was conducted using the Mantel–Haenszel test. The log-rank test was used to analyze the timing of initial ALT normalization, and *t*-test or Wilcoxon test was used for mean ALT values during the treatment and posttreatment observation periods. Significance level was two-sided 5%. All calculations were performed by SAS program version 6.12 (SAS Institute, Cary, NC).

3. Results

3.1. Patient characteristics

The study included 167 patients given combination therapy and 105 assigned monotherapy. Main patient characteristics are shown in Table 1. Mean age was 48–49 years and the majority of patients had extremely high HCV RNA levels exceeding the upper limit of quantitation of 850 kcopies/mL. No imbalance in patient background was observed between the two treatment groups.

3.2. ALT normalization rate

Sustained ALT normalization rate was 28.1% (47/167) in the combination therapy group and 10.5% (11/105) in the monotherapy group; combination therapy was significantly superior to monotherapy ($P = 0.001$; Fisher's direct probability test). Sustained ALT normalization rate taking into account baseline ALT levels is shown in Fig. 1. In this respect, combination therapy was again significantly superior to monotherapy ($P = 0.001$; Mantel–Haenszel test). Sustained ALT normalization rate was also significantly superior in the combination therapy group in patients whose pretreatment ALT was 100 to <150 IU/L ($P = 0.001$; Fisher's direct probability test). In the combination group, the frequency of patients with ALT levels sustained within twice the upper limit of normal (i.e. an index of inhibition of progression to HCC [9,10]) was 34.1% (29/85), 44.7% (17/38), and 25.0% (9/36) in those whose pretreatment ALT was 50 to <100 IU/L, 100 to <150 IU/L, and ≥150 IU/L, respectively; in the monotherapy group the frequency was 22.9% (11/48), 0% (0/23), and 14.3% (4/28), respectively (Fig. 2). Combination therapy was hence significantly superior to monotherapy ($P = 0.001$; Mantel–Haenszel test).

Table 1
Patient demographics at baseline

	IFN + ribavirin	IFN alone	*P*-value
n	167	105	–
Sex (M/F)	135/32	81/24	0.538 (F)
Age (years), mean (S.D.)	47.9 (10.1)	49.1 (9.3)	0.391 (T)
HCV levels (kcopies/mL)			
<500	37	25	0.677 (MH)
500 to <850	46	21	
≥850	84	59	
ALT levels (IU/mL)			
50 to <100	87	48	0.228 (MH)
100 to <150	41	26	
≥150	39	31	
IFN treatment history			
Naïve	39	17	0.060 (MH)
Relapser	82	53	
Nonresponder	40	35	

F, Fisher's exact test; T, *t*-test; MH, Mantel–Haenszel test.

3.3. Association between virological efficacy and sustained ALT normalization

The patients judged to have sustained ALT normalization were divided into SVR and non-SVR patients based on virological efficacy and the effect of the addition of ribavirin to IFN assessed (see Table 2). The sustained ALT normalization

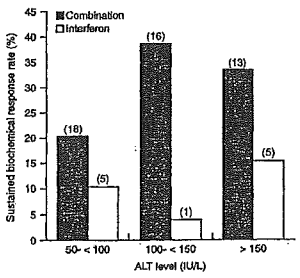

Fig. 1. Rate of sustained biochemical response to treatment by baseline ALT levels. The rate of sustained ALT normalization was significantly better with combination therapy than with interferon monotherapy (Mantel–Haenszel test: $P < 0.01$). Numbers of patients in the parenthesis.

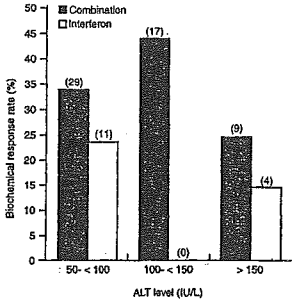

Fig. 2. Rate of patients with ALT levels sustained at less than twice the upper limit of normal (Mantel–Haenszel test: $P < 0.01$). ALT levels were measured ≥4 times during the post-treatment follow-up period. Numbers of patients in the parenthesis.

Table 2
Rate of sustained ALT normalization by virological response at end of follow-up and baseline ALT levels

ALT level (IU/L)	IFN + ribavirin		IFN alone	
	SVR	Non-SVR	SVR	Non-SVR
50 to <100	80% (8/10)	13% (10/77)	–	10% (5/48)
100 to <150	75% (6/8)	30% (10/33)	–	4% (1/26)
≥150	90% (9/10)	14% (4/29)	100% (2/2)	10% (3/29)
Total	82% (23/28)	17% (24/139)	100% (2/2)	9% (9/103)

rate in non-SVR patients was 17.3% (24/139) in the combination therapy group and 8.7% (9/103) in the monotherapy group. The results of the Mantel–Haenszel test taking ALT levels into account showed that combination therapy was significantly superior to IFN alone ($P = 0.034$). Logistic regression analysis determined the factors for sustained ALT normalization in both treatment groups, and are shown in Table 3. The risk for not achieving sustained ALT normalization in nonresponders to previous IFN treatment was four times higher than in IFN-treatment-naïve patients and relapsers. Among non-SVR patients with sustained ALT normalization, low pretreatment WBC count was the only significant influencing factor (data not shown).

3.4. Effect of timing of initial ALT normalization

The timing of initial ALT normalization with respect to pretreatment ALT levels is shown in Fig. 3. Log-rank test

Table 3
Multiple logistic regression analysis of factors associated with sustained ALT normalization

Variables	Odds ratio (adjusted)	95% CI	*P*-value
IFN nonresponder vs. relapser/naïve	0.250	0.093–0.669	0.0148
ALT	1.005	1.001–1.009	0.0116
WBC	1.000	0.999–1.000	0.0416
Serum creatinine	7.959	0.915–69.213	0.0598
IFN relapser vs. nonresponder/naïve	0.558	0.261–1.193	0.1277
Platelet	1.056	0.972–1.148	0.1931

indicated that the timing of normalization was significantly earlier in the combination group of patients with ALT levels ≥100 IU/L. ALT normalization rate at the end of treatment was directly correlated to pretreatment ALT levels: 83.9% (73/87), 80.5% (33/41), and 61.5% (24/39) of those whose baseline ALT was 50 to <100 IU/L, 100 to <150 IU/L, ≥150 IU/L, respectively, in the combination group and 79.2% (38/48), 53.8% (14/26), and 45.2% (14/31), respectively, in the monotherapy group showed normalization. ALT normalization rate at the end of treatment was significantly lower in the monotherapy group versus the combination group ($P = 0.015$; Mantel–Haenszel test).

3.5. Change in ALT levels by virological efficacy

Figs. 4 and 5 show mean ALT values during and after treatment in relapsers and nonresponders, respectively. No significant difference was observed between the combination and monotherapy groups in pretreatment ALT levels in either relapsers or nonresponders. When change in ALT levels during treatment in the monotherapy and combination therapy groups was compared, no difference in effect of HCV RNA-negativity during treatment was observed among relapsers. However, when the two treatment groups were compared at all time points after the end of treatment, ALT values were significantly lower in the combination therapy group ($P < 0.001$; Wilcoxon test). Furthermore, the mean value for all measurement time points was <80 IU/L in this treatment group. Moreover, in virological nonresponders all-time point ALT values were significantly lower in the combination group compared

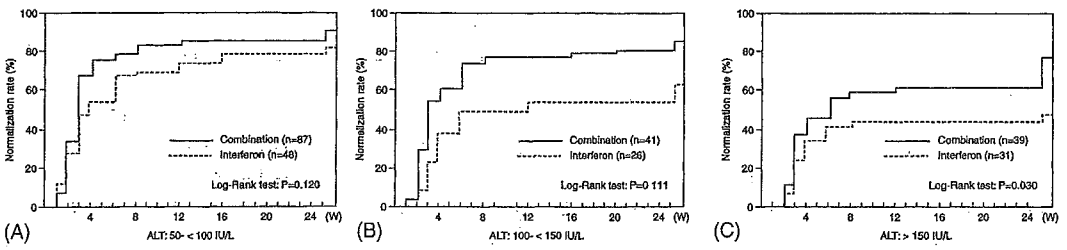

Fig. 3. Initial timing of ALT normalization by pretreatment ALT levels. No significant difference was observed between the combination and monotherapy groups in the timing of initial ALT normalization in patients with low pretreatment ALT levels (50–100 IU/L). In patients with pretreatment ALT levels of ≥100 IU/L, timing of initial ALT normalization was significantly earlier in patients receiving combination therapy than in patients receiving monotherapy.

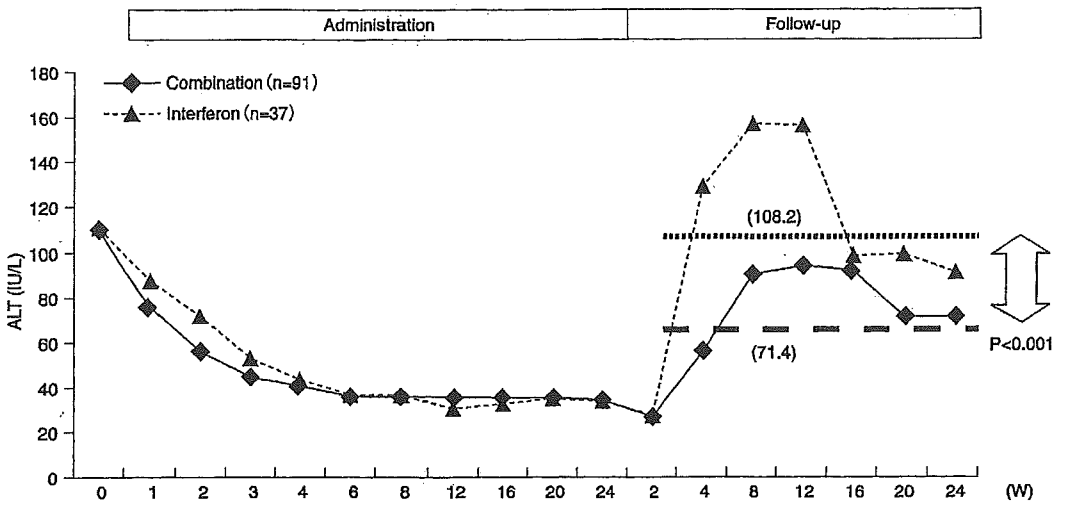

Fig. 4. Changes in ALT levels by viral response: relapsers. No difference was observed between patients receiving combination therapy and monotherapy during the treatment period. After the end of treatment, whereas ALT levels averaged within twice the upper limit of normal in patients receiving combination therapy, a period of marked increase in ALT levels was observed in patients receiving monotherapy. Mean ALT level over the entire period was significantly lower in the combination vs. monotherapy group.

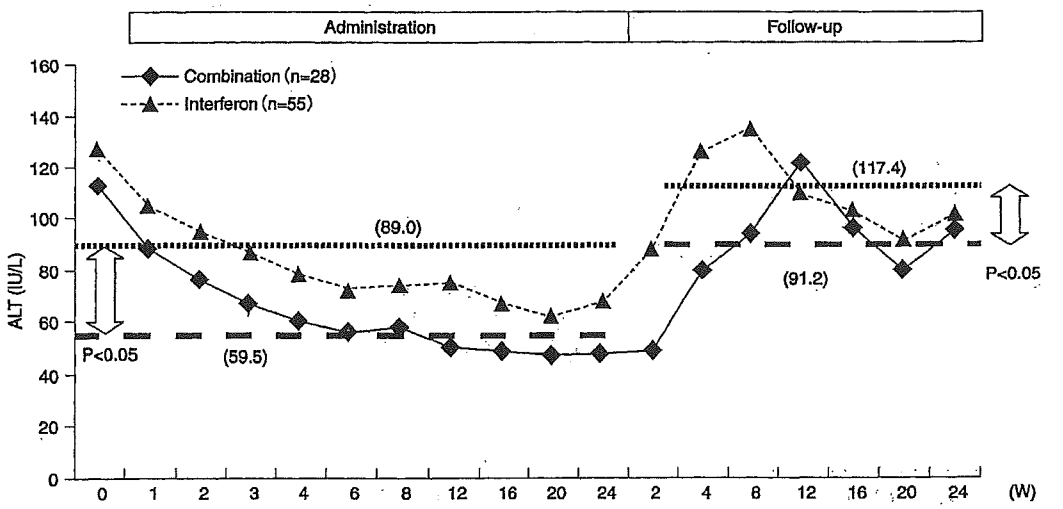

Fig. 5. Changes in ALT levels by viral response: nonresponders. Mean ALT level over the entire period was significantly lower in nonresponder patients receiving monotherapy than in patients receiving combination therapy.

with in the monotherapy group ($P = 0.036$; Wilcoxon test). The mean value for all measurement time points during treatment was <80 IU/L.

4. Discussion

Pretreatment liver function affects progression to liver cirrhosis [24] and gender, alcohol consumption, ALT levels, and histological activity index are factors influencing progression to this condition in HCV-infected patients. This is perhaps related to the observation by Takimoto et al. [8] that achieving SVR in patients with high ALT levels is relatively easy. In the current study, SVR rate was observed to increase in correlation with higher pretreatment ALT levels in both the combination and monotherapy groups (data not shown). On the other hand, Yabuuchi et al. [7] have shown that ALT concentrations in patients with sustained ALT normalization, even in those who become HCV RNA-positive, are significantly lower than in SVR patients and virological nonresponders. In the present study, ALT normalization during treatment occurred at a higher rate in correlation with lower baseline ALT levels, but earlier timing of ALT normalization was not necessarily associated with sustained ALT normalization. However, sustained ALT normalization was achieved more easily in patients with high pretreatment ALT levels regardless of

treatment group. In contrast to non-Japanese reports [25,26], we found that achieving sustained ALT normalization was difficult in nonresponders and relapsers. Older age, longer disease duration, and difference in IFN dose may have been causative factors regarding this result; the reason could not be clarified in this study.

When IFN was first introduced for the treatment of chronic hepatitis C in Japan, the ALT normalization rates reported in genotype 1 patients ranged at about 18–32% [2,3,6]. Many of the patients included in the present study were nonresponders and relapsers to previous treatment, but nevertheless the observed sustained ALT normalization rate (15%) was not very different from those reported previously. In large-scale Japanese clinical studies, the incidence of sustained ALT normalization in non-SVR patients with genotype 1 was about 7–16% [2,4,6,7]. The incidence is estimated about 10% at maximum with IFN monotherapy. The present study not only indicates that addition of ribavirin improves SVR rate but also increases the incidence of sustained ALT normalization including in non-SVR patients compared with IFN monotherapy. Hence sustained liver function normalization was improved by about 30%. Furthermore, ribavirin add-on therapy significantly boosted the number of patients whose ALT was maintained within twice the upper limit of normal, which may reduce the risk of progression to HCC.

An issue that is gaining increasing importance for the future is the method of prevention of progression to HCC in virological nonresponders [27]. In Japan, the long-term rate of hepatocarcinogenesis is considered not greatly different between virological nonresponders to IFN therapy and untreated patients [5,8]. The 5-year incidence of cancer in virological nonresponders has been reported variously at 5–14% [6,7]. Yearly incidence rates of about 1–2% [4,8,28] including a high 5.1% [5] have also been reported. In studies conducted in the USA and Europe, the 5-year incidence rate of liver cirrhosis and HCC in virological nonresponders was 27.8% and 27%, respectively [29,30]. Furthermore, the incidence of progression to HCC in patients with advanced histology followed for 5–7 years was 9.6% [30]. We observed significantly lower on-treatment ALT levels in combination therapy patients versus those on monotherapy, which remained significantly lower after the end of treatment. The timing of ALT flare-up was also delayed in the combination group. This result cannot be explained by differences in change of HCV levels in the two groups since these were not significant (data not shown). However, whether the sustained low ALT levels associated with IFN plus ribavirin combination therapy will lead to less HCC will only be revealed with longer follow-up. Since improved liver function by continued ribavirin monotherapy in virological nonresponders to IFN plus ribavirin has been reported [31], long-term residual effects of ribavirin even after the end of treatment are a possibility. A large-scale clinical study of the effect of long-term treatment with PEG-IFN on prevention of progression to HCC in virological nonresponders to PEG-IFN plus ribavirin combination therapy is ongoing [32]. Long-term IFN monotherapy was reported to enhance ALT normalization in patients without HCV-RNA negative after previous IFN therapy [33].

On the other hand, in patients who relapsed after the end of treatment, mean ALT after the end of treatment was significantly lower in combination versus monotherapy patients. Relapse was observed delayed at 4 weeks after the end of treatment in the combination group (data not shown), suggesting a contribution of HCV to difference in the pattern of change in ALT levels. Viral relapse rate is known to be lowered by long-term combination therapy [12,13,34], and there is much expectation for increased efficacy by this regimen. The results of studies designed to test the hypothesis that time to onset of HCC is prolonged by combination therapy are awaited.

The present study was limited in that while it conclusively demonstrates that combination therapy with ribavirin increases the rate of sustained ALT normalization compared with IFN monotherapy, it was not powered to show prevention of progression to HCC in the long term. Large-scale clinical trials are necessary to examine this postulate. In particular, it is important to determine whether the period of prevention to progression to HCC is extended in virological nonresponders to combination therapy.

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C型慢性肝炎の治療

C型肝炎のIFN反応性とその治療効果予測因子 宿主側要因

Host factor to determine the outcome of IFN treatment for chronic hepatitis C

清澤研道

Key words：宿主因子，肝線維化，インターフェロンレセプター，HLA抗原，抗原提示関連遺伝子多型

はじめに

C型慢性肝炎に対するインターフェロン(IFN)治療効果を規定する因子として，ウイルス側因子，使用薬剤側因子が重要であることが明らかとなっている．ウイルス側因子としてはHCV-RNA量，HCV遺伝子型，NS5Aの遺伝子変異は効果予測に有用である．一方，治療薬としてのIFNの使用量，治療期間，ribavirinとの併用，更にはpolyethylenglycol(PEG)化したIFN製剤の使用などにより治療効果が向上していることから，抗ウイルス剤やその使い方が効果を規定していることは自明の理である[1]．しかしながら，それでもなおHCV遺伝子1b型で高ウイルス量であってもsustained viral response(SVR)を呈する症例や，HCV 2a型や低ウイルス量であってもSVRに至らない症例もある．このことは宿主側に治療効果を規定する因子が存在することを示唆している．

1. 宿主因子

表1にIFN治療効果を予測するに有用な宿主因子を列挙した．HCV感染期間は感染後数年以内のものはHCV 1b型，高ウイルス量でも著効率は高いが，10年以上のものでは，血小板数や肝線維化と関連した因子となる．飲酒は肝内脂肪沈着，線維化，鉄沈着を助長する因子でありリスクファクターである．

表1　ウイルス学的著効を規定する宿主因子

宿主因子	陽性因子	陰性因子
性	女　性	男　性
年　齢	若　年	高　齢
body mass index(BMI)		30以上
飲　酒	禁　酒	多　飲
HCV感染期間	短　い	長　い
肝線維化	少ない	多　い
鉄沈着	少ない	多　い
血小板	多　い	少ない
IFNレセプター発現	多　い	少ない
HLA	表3参照	

body mass index(BMI)が30 kg/m^{2}以上では抗ウイルス治療の抵抗因子であることがretrospective studyから明らかにされた．この場合HCV遺伝子型や線維化(肝硬変)とは独立した因子であるという[2]．BMIが高いと肝細胞に脂肪が沈着し，このことが抗ウイルス剤の吸収，シグナル伝達を障害していると考えられている．更には脂肪沈着は線維化を助長していると考えられている[3]．

Kendo Kiyosawa: Internal Medicine, Shinshu University School of Medicine　信州大学医学部内科(消化器内科)

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表2 肝組織所見，HCV遺伝子型別SVR率[4]

	遺伝子型			
	1b	2a	その他	計
慢性持続性肝炎	11/21(52.4%)	6/7 (85.7%)	0/1 (0 %)	17/29(58.6%)
慢性活動性肝炎(軽症)	19/59(32.2%)	18/21(85.7%)	10/15(66.7%)	47/95(49.5%)
慢性活動性肝炎(重症)	5/61(8.2%)	9/14(64.3%)	2/3 (66.7%)	16/78(20.5%)
肝硬変	2/38(5.3%)	0/2 (0 %)		2/40(5.0%)

肝線維化，インターフェロンレセプター(IFN-R)，HLAについて後述する．

2. 肝組織所見

IFN 600万-1,000万単位を6カ月間投与する標準的な方法では，治療前の肝組織所見はIFN効果に影響を及ぼしている．標準的治療を行い，遺伝子型，肝組織別にSVRをみた飯野らの成績を**表2**に示した[4]．この**表2**での組織所見はstage分類していないが，慢性持続性肝炎はF1，慢性活動性肝炎(軽症)はF2，慢性活動性肝炎(重症)はF3，肝硬変はF4と置き換えることができよう．遺伝子1b型でみると組織所見が治療効果に影響を及ぼしていることが顕著に出ている．肝線維化重症者，特にF4は幾つかの多変量解析においても独立因子としてIFN治療抵抗因子である[5,6]．

3. IFN-R

ウイルス肝炎治療に用いられるIFNαとIFNβはtype I IFN-Rを介して作用する．type I IFN-Rは両者に共通のレセプターである．IFN-RにはIFNαレセプターであるIFNAR1とIFNα/βレセプターであるIFNAR2とがある．

C型慢性肝炎に対するIFN治療効果と肝組織内IFN-Rとの関係には幾つかの報告がある．Fukudaら[7]はIFNAR1について，Yatsuhashiら[8]はIFNAR2について肝組織内mRNAを解析するとともにIFN-Rの発現を検討した結果，いずれもSVRを呈した患者群では発現が多い．Mizukoshiら[9]も同様にIFNAR2のmRNAをcompetitive RT-PCR法で半定量化し，SVR患者においてレセプター発現が多いことを報告している．更にYatsuhashiら[10]は免疫染色法にて肝組織内のIFN-Rの蛋白レベルでの検討も行っているが，やはりSVR群で優位に高いことを報告している．更に，Moritaら[11]はIFNAR1とIFNAR2の2つのレセプターを肝組織内で同時に発現を検討し，SVR例では優位に両者の発現が高いとしている．このように，C型慢性肝炎患者の肝組織内IFN-Rの発現はIFN治療効果に影響を及ぼしていることから，IFN効果を規定する因子となり得る．三代らはIFN産生に関連する遺伝子解析から治療効果に影響する宿主因子を検討した．その結果，IFNR1のプロモーター領域にGTジヌクレオチドリピート多型がありIFN効果予測の宿主因子になると報告した[12]．また，IFNにより誘導される蛋白であるMxAのsingle nucleotide polymorphism(SNP)を検討した研究から，MaX promoterのnt-88のallele G/TがG/Gとなっているのがインターフェロン著効群では31%であるのに比較して非著効群では62%と有意(p=0.0009)に高く，この遺伝子のSNPがIFN効果に影響を及ぼす因子であるとしている[13]．

4. HLA遺伝子およびその多型性

a. HLA抗原

HLA抗原はヒト第6染色体に存在するHLA遺伝子群によりコードされる細胞表面蛋白である．HLAとIFN治療効果の関係をみた報告はそう多くないが，因果関係があるとされているものを**表3**にまとめた．Kikuchiら[14]はB54とA24-B54-DR4ハプロタイプは治療抵抗性であるとした．Alricら[15]はHCV遺伝子1b型ではDQB1*06が治療効果が有意に増し，DRB1*07は治療抵抗性であるとした．Miyaguchiら[16]はB55，B62，CW3，CW4が治療効果を高めるとし，Wawrzynowicz-Syczewskaら[17]はDRB1*

表3 IFN治療効果と関連するHLA

報告者(文献)	治療効果促進HLA	治療抵抗HLA
Kikuchi I[14]		B54 A24-B54-DR4
Alric L[15]	DQB1*06(HCV 1b)	DRB1*07
Miyaguchi S[16]	B55, B62, CW3, CW4	
Wawrzynowicz[17]	DRB1*0701-DQA1*0201-DQB1*02	
Muto H[18]	DR6(-)	

0701-DQA1*0201-DQB1*02ハプロタイプが有用であるとした．教室のMuto[18]はIFN著効群ではDR6が有意に低いことを報告している．HLAは人種によって違いがあり，結果がばらつきのあるのは当然であるが，同じ日本人だけでみても同一のHLAがみられないのは症例数，プロトコールの相違のためかもしれない．また，HLA抗原はクラスI，クラスIIともに遺伝子多型性に富んでおり，HLAのタイプにより結合するペプチドが異なるとされる．したがって個体によってウイルスに対する感受性や免疫応答が異なることが示唆される．一方，これらの報告はいずれもIFN単独の6カ月治療がほとんどであり，長期治療，ribavirinとの併用療法，PEG-IFN治療での検討はいまだなされておらず今後の課題である．

b. HLA抗原提示関連遺伝子多型

Sugimotoら[19]はIFN治療効果とHLAクラスI拘束性の抗原提示関連遺伝子であるTAP1*，TAP2*，LMP2，LMP7の多型性をIFN著効群(n=49)と無効群(n=126)で比較検討したところTAP1*，TAP2*，LMP2の遺伝子頻度の分布に差異は認めなかったが，LMP7-K(アミノ酸部位49 Lys)は著効群において高頻度に認められた(16% vs 7.9%)．更に，LMP7-K，HCV-RNA量，性差，年齢，ALT値，HCV遺伝子型，肝組織像を多変量解析したところ，HCV-RNA($p<0.001$(オッズ比0.40[95%CI 0.24-0.65]))に次ぎLMP7-K($p<0.02$(オッズ比4.5[95%CI 1.4-14]))が独立因子として抽出された(表4)．

表4 IFN著効に寄与する因子の多変量解析[19]

因子	p値	odds ratio (95%CI)
LMP7(K vs Q)	0.0011	4.5(1.4-14)
LMP2(H vs R)	NS	
TAP1*(0101, 0201, 0301, 0401)	NS	
TAP2*(0101, 0102, 0103, 0201)	NS	
HCV-RNA titre	0.0003	0.40(0.24-0.65)
sex(male vs female)	NS	
age	NS	
ALT	NS	
genotype(1b vs 2a or 2b)	NS	
histological stage (mild vs moderate or severe)	NS	

このことはIFN治療による著効にHLAクラスI拘束性の抗原提示関連遺伝子であるLMP7遺伝子の多型性が関与していることが示唆されている．

5. ケモカイン

インターロイキン10のプロモーターの遺伝子多型[20]やケモカイン受容体の一種であるCCR5のプロモーターSNPs(59029-A allele)[21]がC型慢性肝炎の治療効果に関係するという報告がある．

おわりに

C型慢性肝炎に対する抗ウイルス療法に及ぼす宿主因子は治療法の進歩により，より真実が明らかにされるであろう．

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