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Abstract

To improve the efficacy of interferon (IFN) treatment for chronic hepatitis C, we have proposed the twice-daily administration of IFN-beta as a promising induction therapy. In this study, we demonstrated differences between the clearance of circulating HCV-RNA and the induction of anti-viral actions during the first 2 weeks of treatment. Nine patients with a high viral load and genotype 1b were randomly assigned to 3 groups: group A received 3MU of IFN-beta twice a day at intervals of 5 and 19 h; group B received 3MU of IFN-beta twice a day at intervals of 10 and 14 h; group C received 6MU of IFN-alpha once a day with ribavirin. The expression of OAS2, PKR, and MxA in peripheral blood mononuclear cells (PBMCs) were quantified by real-time polymerase chain reaction method. The viral clearance showed a bi-phasic pattern, and those in the second phase of groups A and B were significantly steeper than that of group C. The peak level of OAS2 during the first phase was correlated with the first phase decay. The MxA expression tended to be higher in group A and B than in group C. The expression of these 3 proteins tended to decrease at day 6 in group C, but increase in groups A and B. These might make differences in the viral decay during the second phase

KEYWORDS: chronic hepatitis C(CHC), interferon(IFN)beta, hepatitis C virus(HCV)dynamics, antiviral actions, real time PCR

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Original Article

Anti-viral Actions and Viral Dynamics in the Early Phase of Three Different Regimens of Interferon Treatment for Chronic Hepatitis C: Differences between the Twice-daily Administration of Interferon-beta Treatment and the Combination Therapy with Interferon-alpha Plus Ribavirin

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To improve the efficacy of interferon (IFN) treatment for chronic hepatitis C, we have proposed the twice-daily administration of IFN-beta as a promising induction therapy. In this study, we demonstrated differences between the clearance of circulating HCV-RNA and the induction of anti-viral actions during the first 2 weeks of treatment. Nine patients with a high viral load and genotype 1b were randomly assigned to 3 groups: group A received 3MU of IFN-beta twice a day at intervals of 5 and 19 h; group B received 3MU of IFN-beta twice a day at intervals of 10 and 14 h; group C received 6MU of IFN-alpha once a day with ribavirin. The expression of OAS2, PKR, and MxA in peripheral blood mononuclear cells (PBMCs) were quantified by real-time polymerase chain reaction method. The viral clearance showed a bi-phasic pattern, and those in the second phase of groups A and B were significantly steeper than that of group C. The peak level of OAS2 during the first phase was correlated with the first phase decay. The MxA expression tended to be higher in group A and B than in group C. The expression of these 3 proteins tended to decrease at day 6 in group C, but increase in groups A and B. These might make differences in the viral decay during the second phase.

Key words: chronic hepatitis C (CHC), interferon (IFN) beta, hepatitis C virus (HCV) dynamics, antiviral actions, real time PCR

I nfection with hepatitis C virus (HCV) is a leading cause of chronic liver disease throughout the world [1], which leads to liver cirrhosis and hepatocellular carcinoma at dramatically elevated rates [2]. Interferon (IFN), a multifunctional cytokine with various biological properties [3], has been widely used for the treatment of chronic hepatitis C (CHC) [4]. However, treatment with IFN-alpha mono-therapy has remained far from satisfactory to date, especially in the case of patients with HCV genotype 1 and a high viral load [5]. Although a combination therapy with IFN-alpha plus ribavirin, which is a synthetic analog and possesses broad

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anti-viral effects against both DNA and RNA viruses, Pegylated IFN-alpha, and the prolongation of treatment have been attempted in order to overcome this limitation of clinical outcomes, they have not proven to be successful [6, 7].

IFN-beta has also been widely used for the treatment of chronic hepatitis C in Japan [8]. Okushin *et al.* reported that the twice-daily administration of IFN-beta treatment for the first 4 weeks of therapy as an induction one followed by IFN-alpha 2b mono-therapy showed a higher sustained virological response rate than a once-daily administration of IFN-beta or an IFN-alpha mono-therapy [9]. Although there have been a number of reports maintaining the superiority of the twice-daily administration of IFN-beta treatment [10–13], there is a little information evaluating the mechanisms of its enhanced anti-viral actions [14].

As regards predicting the long-term clinical effects of IFN treatment, the analysis of serum HCV-RNA decline during the first 2 weeks of treatment (HCV-dynamics) have been reported to be very beneficial [15]. In these reports, the initial decline of the viral load has typically been characterized by a convex curve. The first phase (day 1) of the initial decline tended to be rapid and dose dependent, whereas the second phase ($>$ day 2) is highly variable and shows a much slower decline. Numerous reports have described HCV dynamics and emphasized the usefulness of the early clearance of HCV-RNA during the IFN-alpha therapy [16, 17]. However, few reports have discussed the effects of IFN-beta in great detail [11, 12].

The IFN systems are activated in response to viral infections through the receptor mediated signal transduction pathways, and play an important role in host defense responses [3]. Type I IFNs induce various kinds of proteins with anti-viral activity, such as MxA-proteins (MxA), 2'-5'-oligoadenylate synthetase (OAS), or the dsRNA-dependent protein kinase (PKR)[18, 19]. These 3 proteins are considered to be indicators of IFN actions and are responsible for the anti-viral state induced by the presence of IFN [20].

In this paper, we report a comparative study to evaluate the differences in HCV dynamics and the anti-viral actions during the first 2 weeks of the twice-daily administration of IFN-beta treatment. In our previous *in vitro* study, anti-viral actions induced by IFN-beta were demonstrated to be strengthened the most at the time point of 5 to 6 h after administrations [14]. Therefore, we designed 2 therapeutic schedules with different injection intervals, *i.e.*, 5 h and 10 h, to evaluate the mechanisms of enhanced anti-viral actions induced by the twice-daily administration of IFN-beta treatment and to confirm the hypothesis that the therapy at intervals of 5 h would activate the anti-viral actions more than that of 10 h. In addition, we also examined a made of the combination therapy with IFN-alpha plus ribavirin, which has been a current standard therapy for patients with CHC [6], in order to compare the differences between IFN-alpha and IFN-beta treatment.

Materials and Methods

Patients.   After written informed consent, 9 patients with chronic hepatitis C were enrolled in this

Table 1   Clinical characteristics of patients

	Interferon therapy			*P* value
	Group A*	Group B*	Group C*	
Gender (male/female)	3/0	3/0	1/2	NS
Age	42.2 ± 9.4	53.3 ± 8.6	55.3 ± 8.5	NS
Histological fibrosis (F1/2/3/4)	2/1/0/0	1/1/1/0	0/1/2/0	NS
Histological activity (A1/2/3)	2/1/0	1/2/0	1/2/0	NS
ALT (IU/L)	47.7 ± 14.2	75.3 ± 10.5	90.3 ± 22.8	NS
HCV-RNA (KIU/ml)	760 ± 139	642 ± 200	723 ± 219	NS
Previous IFN therapy (naïve/relapser)	3/0	2/1	1/2	NS

Data were presented as mean ± SD. NS, statistically not significant.
*: Group A, 3 MU of IFN-beta twice a day at intervals of 5 and 19 h; Group B, 3 MU of IFN-beta twice a day at intervals of 10 and 14 h; Group C, 6 MU of IFN-alpha-2b once a day with ribavirin.

study (Table 1). All patients had HCV genotype 1b and a high viral load. Genotypes of HCV were identified by PCR using type-specific primers from the core region, as previously described by Okamoto *et al.* [21]. We considered a high viral load as more than 200 KIU/ml using a quantitative RT-PCR assay (Amplicor HCV Monitor assay, SRL, Tokyo, Japan). A liver biopsy was performed within 12 months before treatment, and its histological findings were classified according to the New Inuyama classification, in which the grades of fibrosis were classified from F0 to F4, and the activity from A0 to A3 [22]. Patients were excluded if they were positive for hepatitis B surface antigen, had histological cirrhosis, or had a history of excessive alcohol consumption. Further exclusion criteria were a history or evidences of autoimmune disease; clinically severe cardiac, hematologic, metabolic, renal, rheumatologic, neurologic; or psychiatric disease; or evidence of interstitial pneumonitis. The study protocol conformed to the Helsinki Declaration of 1975.

Interferon therapy. Nine patients were randomly assigned to 3 groups of different IFN treatment regimens for the first 2 weeks of induction therapy, as follows: group A received 3 MU of natural IFN-beta (Feron, Toray, Tokyo, Japan) twice daily at intervals of 5 and 19 h (at 9:00 and 14:00); group B received 3 MU of IFN-beta twice daily at intervals of 10 and 14 h (at 9:00 and 19:00); and group C received 6 MU of IFN-alpha (Intoron A, Schering-Plough, Kenilworth, NJ) once daily with ribavirin (600 or 800 mg per day; Rebetol, Schering-Plough). In groups A and B, 3 MU of IFN-beta were diluted in 100 ml of 5% glucose solution and administrated intra-venously for 30 min. Each group received 6–10 MU of IFN-alpha 3 times weekly for 22 weeks, followed by the treatment described above.

Sampling of blood. Blood samples were collected from the patients according to the following schedule: on Day 0 and Day 6 at 9:00 (before IFN administration), 14:00, and 19:00; on Day 1 and Day 7 at 0:00 and 9:00; on Day 2 and Day 13 at 9:00 (T = 0, 5, 10, 15, 24, 48, 168, 173, 178, 183, 192, 336 h); on the final day of treatment and after 6 months of follow-up observation. Serum samples were collected from 3–5 ml of peripheral whole blood and stored at $-80\ °C$ until use. Peripheral blood mononuclear cells (PBMCs) were also collected from 10 ml of heparinized peripheral whole blood at the same time. PBMCs were isolated by the Ficoll density gradient centrifugation method (Ficoll-Paque:

Pharmacia Biotech), as reported previously [23] and were also stored in sterile stock tubes at $-80\ °C$ until use.

Quantification of HCV-RNA in serum. Serum HCV-RNA was quantified using the Amplicor HCV monitor assay (SRL, Tokyo, Japan), and the detection limit ranged from 0.5 KIU/ml to 850 KIU/ml.

Calculation of the viral decay slope. The calculation of the viral decay slope was carried out according to the mathematical model previously described [15]. The viral exponential decay slope during the first phase was calculated using the data obtained between pretreatment viral load (T = 0) and the viral load at 48 h after initiation of IFN (T = 48); the log-linear regression test was used for these calculations. The slope during the second phase was calculated using the data obtained from T = 48 to T = 336.

Quantification of the gene expressions of 3 anti-viral proteins in PBMCs. RNA was extracted from PBMCs using the acid guanidinium thiocyanate phenol chloroform (AGPC) method [24], and cDNA was synthesized using RAV-2 (Takara) and random primer (9mer, Takara), according to the manufacturer's instructions. In this study, we chose OAS2 as the representative of 2'5'-OAS, PKR, and MxA as markers of the response to interferon therapies, and GAPDH as an internal control. Gene expressions in PBMCs were quantified by a LightCycler System (Roche) using Hybridization Probes, as described in Table 2. The following protocols were used for the quantification: one cycle of an initial denaturation step at 95 °C for 10 min; 40 cycles of amplification steps consisting of denaturation at 95 °C for 15 sec, annealing at 60 °C for 15 sec, and extension at 72 °C for 5 sec in PKR and MxA, 9 sec in OAS2, and 12 sec in GAPDH. The temperature transition rate was fixed at 20 °C/sec. The amplification was performed in 18 μl of LightCycler DNA Master Probe mix (Qiagen) containing 3 mM MgCl$_2$, 0.5 μM of forward and reverse primer, 0.2 μM of fluorescein probe and 0.4 μM of LC Red probe, and 2 μl of each sample cDNA.

Statistical analysis. Differences between the 3 groups were assessed by a χ^2 test, Fisher's exact test, unpaired t-test, Spearman's correlation and coefficient test, and Mann-Whitney U test. Kruskal-Wallis test, repeated one-way ANOVA, and Fisher's PSLD were used for multi group comparisons. Factors with $P < 0.05$ were considered significant.

Table 2   The sequences of primers and probes

OAS2	Forward primer	5'-GAG TGG AGT GCC GGA TTT T-3'
	Reverse primer	5'-AGA TGC CAA CAC CAA CGG-3'
	Fluorescein probe	5'-TAC AAC TTT GAA GAT GAG ACC GTG AGG-3'-Fluorescein
	LC Red probe	5'-LCRed640-AGT TTC TAC TGA GCC AGT TGC AGA AAA C-3'-phosphorylation
	Amplicon size	225 bp
PKR	Forward primer	5'-CGA TAC ATG AGC CCA GAA AC-3'
	Reverse primer	5'-GTT TCA AAA GCA GTG TCA C-3'
	Fluorescein probe	5'-TTC AGC AAG AAT TAG CCC CAA AGC-3'-Fluorescein
	LC Red probe	5'-LCRed640-TAG AGG TCC ACT TCC TTT CCA TAG TCT TG-3'-phosphorylation
	Amplicon size	116 bp
MxA	Forward primer	5'-AGC TCG GCA ACA GAC TCT T-3'
	Reverse primer	5'-CTG GAG CAT GAA GAA CTG-3'
	Fluorescein probe	5'-TGA TGC CCT ATC ACC AGG AGG C-3'-Fluorescein
	LC Red probe	5'-LCRed640-AGC AAG CGC ATC TCC AGC CA-3'-phosphorylation
	Amplicon size	120 bp
GAPDH	Forward primer	5'-TGA ACG GGA AGC TCA CTG G-3'
	Reverse primer	5'-TCC ACC ACC CTG TTG CTG TA-3'
	Fluorescein probe	5'-TCA ACA GCG ACA CCC ACT CCT-3'-Fluorescein
	LC Red probe	5'-LCRed 640-CAC CTT TGA CGC TGG GGC T-3'-phosphorylation
	Amplicon size	307 bp

bp, Base pair.

Results

Characteristics of patients.   There was no significant difference among the 3 groups with respect to gender, age, histology, alanine amino-transferase activity (ALT), and HCV-RNA (Table 1). All patients in the 3 groups were considered to have HCV genotype 1b and a high viral load, at least 450 KIU/ml by an amplicor HCV monitor assay. One patient from group A discontinued IFN therapy on day 90 due to an economical reason, and another patient from group C discontinued on day 70 due to severe leukopenia.

Kinetics of circulating HCV-RNA decline. HCV dynamics showed a biphasic pattern consisting of a rapid decrease within 48 h after the initiation of the treatment (first phase), and a subsequent slow decrease (second phase) (Fig. 1). All patients showed rapid decreases during the first phase, and the calculated decay slopes did not reveal significant differences between the 3 groups (Table 3). On the other hand, the exponential decay slope of group C during the second phase was significantly more gradual than those of groups A and B ($P = 0.049$ and $P = 0.046$: Table 3).

No patient was negative for serum HCV-RNA at 48 h after the first administration of interferon. The negativity
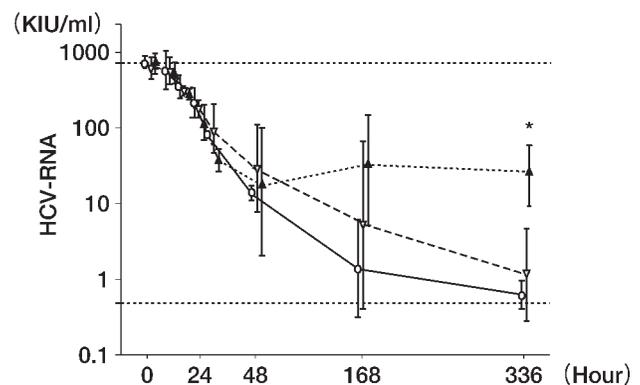

Fig. 1   Mean and SD of log viral load during the first 13 days of 3 different interferon treatments. HCV-RNA ranged from 0.5 to 850 KIU/ml was able to be quantitated. The 3 different regimens include: group A, IFN-beta twice a day at intervals of 5 h (○); group B, IFN-beta twice a day at intervals of 10 h (▽); group C, combination therapy with ribavirin plus IFN-alpha (▲). *At the time of 336 h, which means day 13, statistically significant differences were found between group A *vs*. C and group B *vs*. C ($P = 0.049$, 0.046).

at day 13 (T = 336) was 2/3 in group A, 2/3 in group B and 0/3 in group C. Although all of the patients in this study became negative for HCV RNA at the end of treatment, all of them relapsed.

Table 3   Kinetics of HCV-RNA during interferon therapy

	Interferon therapy			P value
	Group A*	Group B*	Group C*	
Exponential decay slope (log10/day)				
First phase	0.896 ± 0.079	0.696 ± 0.181	0.896 ± 0.234	0.561
Second phase	0.104 ± 0.016	0.113 ± 0.017	$-0.024 \pm 0.037*$	0.063

Data were presented as mean $\pm$ SD. Exponential decay slope was calculated by the linear regression test.
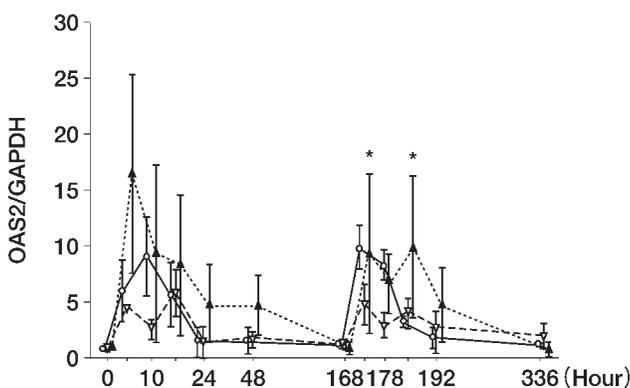*$P = 0.0494$ *vs.* Group A and $P = 0.046$ *vs.* Group B.

Fig. 2   The gene expression levels of OAS2 during the first 13 days of 3 different interferon treatments. Data (mean and SD) are presented as the increment ratio of OAS2 between each point and hour 0. Group A was presented as (○), group B (▽) and group C (▲). *Statistically significant differences in each time point were observed at T = 178 (Group A *vs.* B: $P = 0.045$) and T = 183 (Group A *vs.* C: $P = 0.025$).
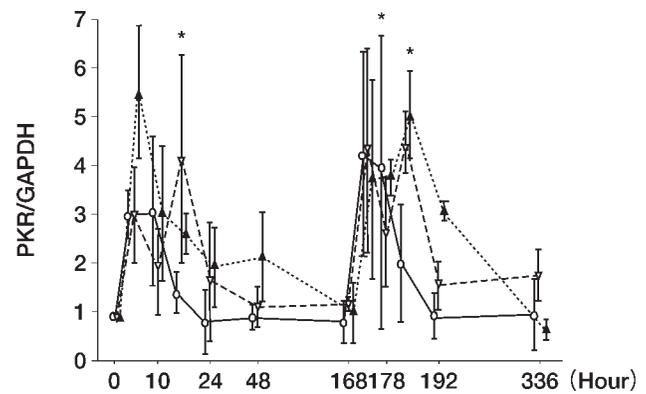

Fig. 3   The gene expression levels of PKR during the first 13 days of 3 different interferon treatments. Data (mean and SD) are presented as the increment ratio of PKR between each point and hour 0. Group A was presented as (○), group B (▽) and group C (▲). *Statistically significant differences were observed at T = 15 (Group A *vs.* C: $P = 0.048$), T = 178 (Group A *vs.* C: $P = 0.043$) and T = 183 (Group A *vs.* C: $P = 0.028$).
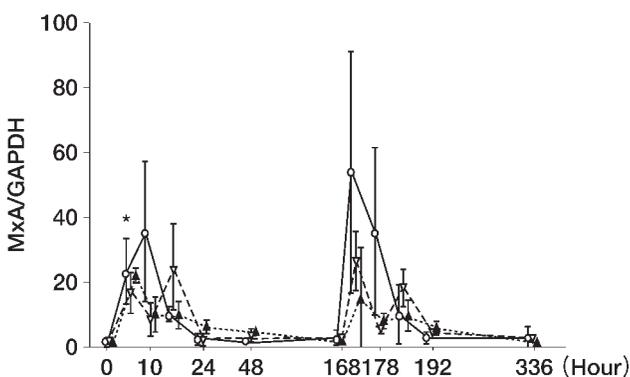

Fig. 4   Mean and SD of the gene expression levels of MxA during the first 13 days of 3 different interferon treatments. Data are presented as the increment ratio of MxA between each point and hour 0. Group A was presented as (○), group B (▽) and group C (▲). *Statistically significant differences were observed at T = 5 (Group B *vs.* C: $P = 0.035$).

Gene expressions of anti-viral proteins.
After the initiation of IFN treatment, the gene expressions of 3 anti-viral proteins in PBMCs were shown to have been induced in similar patterns (Figs. 2, 3, 4). The peak levels during the first phase were observed at T = 10 in group A, and T = 15 in group B, and T = 5 in group C (Table 4). In the second phase of viral decline, the anti-viral proteins were induced at T = 173 in group A, and at T = 173 or later in groups B and C (Table 4). The peak level of OAS2 during the first phase was the highest in group C. Although there were some differences in the expression of these anti-viral proteins during the study at several time points among the 3 groups, there was no significant relation between IFN protocols and the overall expressions of 3 anti-viral proteins in the second phase. However, MxA tended to be most highly expressed in group A, then group B, and was least

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Table 4   Peak level of gene expression of anti-viral factors

Anti-viral factors*	Interferon therapy			*P* value
	Group A*	Group B*	Group C*	
OAS2, the first phase	9.32 ± 3.53 (T = 10)	6.06 ± 1.99 (T = 15)	16.4 ± 8.70 (T = 5)	0.079
the second phase	10.03 ± 1.93 (T = 173)	5.12 ± 1.76 (T = 173)	10.03 ± 6.30 (T = 183)	0.28
PKR, the first phase	3.51 ± 1.74 (T = 10)	4.74 ± 0.48 (T = 15)	5.51 ± 1.36 (T = 5)	0.39
the second phase	4.85 ± 2.40 (T = 173)	5.10 ± 0.74 (T = 183)	3.76 ± 0.36 (T = 178)	0.53
MxA, the first phase	35.5 ± 21.7 (T = 10)	24.6 ± 9.25 (T = 15)	22.0 ± 2.35 (T = 5)	0.73
the second phase	53.9 ± 37.2 (T = 173)	26.7 ± 9.25 (T = 173)	14.5 ± 16.4 (T = 173)	0.20

*, Peak levels were presented as the increment ratio to pre-treatment levels. Numbers in parenthesis indicate hours at peak levels.
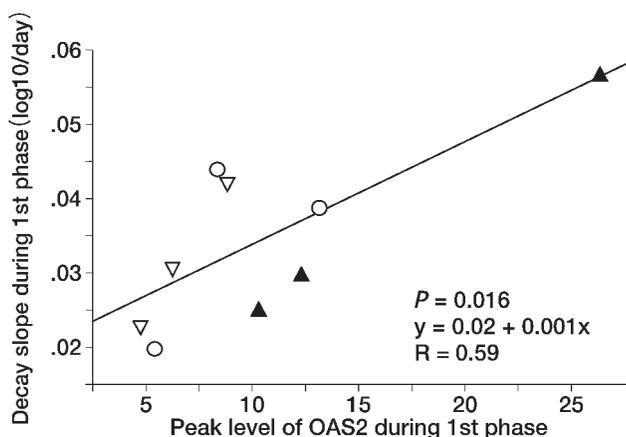

Fig. 5   Regression analysis of the correlation between the peak levels of OAS2 and HCV-RNA exponential decay slopes during the first phase. Statistical analysis was done by Spearman's correlation coefficient test. OAS2 value was presented as the increment ratio. Group A was presented as (○), group B (▽) and group C (▲). The peak level of OAS2 during the first phase was correlated with the first phase decay statistically.

PKR and MxA ($P = 0.40$ and 0.68, data not shown). As regards the duration of activated anti-viral status as it related to the viral decay slopes, no conspicuous tendency was observed.

Discussion

Viral kinetics observed with IFN treatment revealed that the slope of the first phase of viral decay was steep and considered to be related to the inhibition of HCV replication induced by IFN and mainly reflected the degradation rate of free viruses [15]. The second phase of the viral decay slope was gradual and was considered to be related to the degradation rate of the infected cells, which was correlated to the clinical outcome. In our study, there was no significant difference in the viral decay of the first phase among the 3 groups. On the other hand, groups A and B, which received IFN-beta twice daily, showed much steeper decline slopes during the second phase than did group C (Fig. 1; $P = 0.019$), and these data were almost consistent with the previous reports [12]. Therefore, it could be concluded that the induction therapy by a twice-daily administration of IFN-beta had a possibility to improve the clinical outcomes [9–11].

Benefits by introducing twice-daily IFN-beta administration were discussed in several studies [9, 10, 13], and the pharmacological and immunological mechanisms of such treatment were also addressed in those reports. First, the presence of a second-receptor associated protein may involve in the signal transduction pathway induced only by IFN-beta, although IFN-alpha and IFN-beta share common cell surface receptors [25]. Second, the serum level of IFN-beta could be maintained within a therapeutic range by administrated twice daily. However,

expressed in group C. In addition, the peak levels of these 3 anti-viral proteins in group C on day 6 tended to be less than those on day 0, but this tendency was not observed in groups A and B (Fig. 4 and Table 4).

The relation between HCV decay slopes and the expression of anti-viral protein induced by IFNs.   We compared pre-treatment levels and peak levels of each anti-viral protein during the first phase to the exponential decay slopes of HCV-RNA during the first and second phases. The peak level of OAS2 during the first phase correlated statistically with the first phase decay ($P = 0.016$, R = 0.59: Fig. 5). However, this difference was not as clear in the case of

we think this might be less possible. Because the serum level of IFN-beta declined undetectable within 1 h after administration and remained within the undetectable range throughout the therapy, when IFN-beta was injected intra-venously, [11, 26]. Third, our previous study suggested that the enhancement of the anti-viral response induced by the twice-daily treatment of IFN-beta was due to the priming effect of IFN [13, 14].

In vitro study using cell culture systems [14] suggested the treatment of IFN beta twice in an interval of 5 h could induce better anti-viral response than that of 10 h. Thus, we applied this IFN schedule in a clinical setting. In the present study, it was found that the intervals of 5 and 19 h (group A) and 10 and 14 h (group B) provided similar effects on the viral decline slopes and the anti-viral actions. It remains unclear whether or not more frequent dosing of IFN-beta would have contributed to better clinical results, because it has been reported that the high dose administration of IFN reduces the IFN receptor of target cells, and it takes long time to recovery its function [27].

Type I IFN activates multiple signals and the expression of a wide variety of genes, which may contribute to its anti-viral properties [3]. In this study, 3 major anti-viral proteins (OAS2, PKR, MxA) were extensively examined. Although ribavirin was added only in the case of group C, there was no evidence of a difference among pharmacokinetic parameters between those who received the mono-therapy and the combination therapy with ribavirin [28]. The evaluation of the expression level of these gene expressions in liver cells would be more helpful to more precisely understand the reactions involved in the presence of type I IFNs. However, in this study, we focused on the gene expression levels in PBMCs, due to the ethical difficulty of performing repeated liver tissue samplings in human patients.

The 2'5'-OAS system activates RNAse-L and leads to the interferences of viral replication (RNA degradation) [20]. The peak levels of OAS2, which served as the representative of 2'5'-OAS in the present study, were similar to those of previous reports in terms of the increment ratio observed among the 3 groups [29]. Some previous reports have suggested that the pre-treatment serum levels of 2'5'-OAS activity in patients with HCV were significantly higher than those in healthy controls with differences among individuals [30–32], and in this study as well, the pre-treatment level of OAS2 ranged from 0.520 to 1.71 (expressed as OAS2/

GAPDH; data not shown). In addition, previous reports have indicated that the activation of 2'5'-OAS had no significant relationship with a sustained virological response to IFN therapy, although there were tendencies [30–33]. Such findings might reflect viral resistances to the anti-viral activities of host cells. In this study, the peak level of OAS2 during the first phase was statistically correlated with the first phase decay ($P = 0.016$, R = 0.59: Fig. 5), but the duration of the activated status of OAS2 was not. When we considered the difference of the antiviral gene activation after 1 week of interferon treatment, we observed that the peak levels of OAS2 in group C on day 6 were tended to be lower than those on day 0, but this was not the case in groups A and B (Table 4). This finding might reflect the differences between the viral decay slopes obtained during the second phase, which were likely due, at least in part, to the down-regulation of the type I IFN receptor.

PKR is known to inhibit translation initiation through the phosphorylation of protein synthesis initiation factor eIF-2 alpha, and it is also known to regulate transcriptional activation through activating nuclear factor (NF)-κB (mRNA translation inhibition) [20]. The expression of the PKR was not shown to differ among the 3 groups of this study, and no correlation between gene expression levels of PKR and the viral decay slope was determined. Moreover, MxA expression levels were not correlated with the viral decay slopes. However, in the second phase, MxA protein expression tended to be induced more vigorously by twice-daily IFN-beta treatment than by IFN-alpha plus ribavirin treatment (Fig. 4, Table 4). Mx proteins possess GTPase activity, and they also appear to target viral nucleocapsids and inhibit RNA synthesis [20]. A previous report has indicated that the long-term MxA induction was greater in sustained virological responders than in non-responders, although the difference between the 2 was not statistically significant [32]. Another report suggested that patients not showing MxA mRNA enhancement after the initiation of IFN therapy might become non-responders [34]. Further studies may reveal the relationship between these anti-viral proteins induced by IFNs and clinical outcomes.

In summary, we demonstrated differences in HCV-dynamics and anti-viral actions induced by type I IFNs in patients with a high viral load and genotype 1b, who were treated with the combination therapy with IFN-alpha plus ribavirin or the twice-daily intravenous administration of IFN-beta. Twice-daily IFN-beta treatment was shown to

produce steeper viral decay slope than those observed with the combination therapy. As regards anti-vial actions, the peak level of 2'5'-OAS correlated with the first phase decay slope among the 3 groups. This correlation was somewhat equivocal in the case of PKR and MxA in this study. MxA protein expression tended to be induced more vigorously in the twice-daily IFN-beta groups than in the combination group. The activation of each gene on day 6 was less than that observed on day 0 only in group C, and this finding might be associated with differencse in the viral decay during the second phase. Anti-viral actions might be enhanced differently by different IFN protocols in patients with HCV, further studies must be conducted to identify the unknown mechanisms involved in the IFN treatment of patient with HCV, and to improve the clinical outcomes.

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