In the present study, we sought to elucidate the underlying mechanism of the enhanced antiviral efficacy seen with PEG-IFN alpha-2b and ribavirin combination therapy by analysing PKR gene expression and pharmacokinetics of PEG-IFN and ribavirin in patients with chronic HCV genotype 1b infections. The relationships between the viral response and PKR expression and pharmacokinetics of PEG-IFN and ribavirin were also studied.

MATERIALS AND METHODS

Patients

Fifty-two patients infected with chronic hepatitis C of genotype 1b and high viral load, admitted between November 2001 and June 2002, were included in the study. Twenty-six patients were treated with PEG-IFN alpha-2b and ribavirin combination therapy, with the remaining 26 patients matched for age, body weight and dose of ribavirin being treated with conventional IFN alpha-2b and ribavirin. The inclusion criteria for the study were as follows: Persistent elevation of serum alanine aminotransferase (ALT) levels above the upper limit of the normal for ≥6 months prior to therapy; the presence of HCV genotype 1b in the serum; the presence of serum HCV-RNA of >100 000 IU/mL detected by the Amplicor-HCV monitor assay (Roche Molecular Diagnostic Co., Tokyo, Japan); no evidence of hepatocellular carcinoma in an ultrasound examination; a haemoglobin level ≥14 g/dL, neutrophil count ≥1500/mm^3, platelet count ≥100 × 10^3/mm^3, creatinine clearance ≥51 mL/min and fasting blood sugar <110 mg/dL. Exclusion criteria included the presence of hepatitis B surface antigen or human immunodeficiency viral antibodies and a history of excess alcohol consumption. Eleven of the 26 PEG-IFN alpha-2b recipients and all 26 conventional IFN alpha-2b recipients had been enrolled previously in a viral dynamics study [7].

Written informed consent was obtained from all the patients and the study protocol was approved by the institutional ethical committee in accordance with the revised version of the Helsinki Declaration of 1983.

Treatment

Twenty-six patients were treated for 48 weeks with subcutaneous injections of PEG-IFN alpha-2b (PegIntron®; Schering-Plough Corporation, Kenilworth, NJ, USA) at a dose of 1.5 μg/kg/week. Ribavirin (Rebetol®, Schering-Plough Corporation) was administered concomitantly over the 48-week period, provided orally twice daily at a total daily dose of 800 mg. At the start of the study, 400 mg of ribavirin was administered, with serum concentrations being measured after 48 h. As the body weight of the patients in the study ranged between 60 and 80 kg, the dose of ribavirin for the remainder of the study period was fixed at 800 mg/day. The dose of PEG-IFN alpha-2b was reduced to 0.75 μg/kg/week when either the neutrophil count was <750/mm^3 or the platelet count was <80 × 10^3/mm^3. The dose of ribavirin was reduced to 600 mg/day when the haemoglobin concentration decreased to <10 g/dL.

The remaining 26 patients were treated for 48 weeks with intramuscular IFN alpha-2b (Intron-A®; Schering-Plough Corporation) in combination with daily oral ribavirin at a dose of 800 mg. For the first 2 weeks of therapy, 6 MU of IFN alpha-2b was administered daily, followed for the next 46 weeks by 6 MU given three times a week.

Measurement of PKR mRNA before and during therapy

Serial measurements of PKR expression before and during treatment were determined in both treatment groups. Peripheral blood mononuclear cells (PBMCs) were obtained from whole blood samples collected before, and at 4, 8, 24 h and 2, 4, 7, 14, 21, 28, 56, 84, 112, 140, 168 and 336 days after the initiation of either PEG- or conventional IFN alpha-2b and ribavirin combination therapy. After extraction of total RNA from the PBMCs, the expression of PKR mRNA was quantified at each specified time point using real-time quantitative polymerase chain reaction (PCR) as described previously [8]. The assays were performed in triplicate, and as an internal control, the expression levels of PKR transcript were normalized to glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene expression quantified by real-time quantitative PCR. The level of PKR gene expression at each time point during IFN treatment was calculated relative to baseline expression levels measured prior to IFN treatment.

Pharmacokinetics of pegylated interferon alpha-2b and ribavirin

The pharmacokinetics of PEG-IFN and ribavirin was analysed in 15 PEG-IFN alpha-2b recipients who consented to be enrolled in the additional pharmacokinetic study. Of these 15 patients, two were naïve , nine had relapsed and four had not responded to previous conventional IFN monotherapy. Blood samples were collected immediately before, and at 2, 4, 6, 8, 10, 12, 14, 16, 24, 36, 48, 72, 96, 120, 144 and 168 h after the first dose of PEG-IFN alpha-2b and ribavirin. Blood samples were also collected immediately before each administration at weeks 5, 9, 13, 25 and 37 and the trough values measured. At week 48 (final dose), blood was drawn immediately before, and at 2, 4, 6, 8, 10, 12, 14, 16, 36, 48, 72, 96, 120, 144, 168, 366, 504 and 672 h after administration. The sera were harvested immediately after blood collection and stored frozen at −20°C.

Serum PEG-IFN alpha-2b levels were determined using an electrochemiluminescent immunoassay (IGEN International, Inc., Gaithersburg, MD, USA), with the lower limit of detection for this assay being 27 pg/mL. Serum ribavirin levels were measured by high-performance liquid chromatography

in conjunction with tandem mass spectroscopy (MDS Pharma Services Inc., Montreal, QC, Canada) according to a method reported previously [9]. The maximum serum concentration (C_{max}), time to maximum serum concentration (t_{max}) and C_{168h} (trough value of ribavirin) were then determined. Confirmation of the steady state using circadian changes of the trough value, estimation of the time to reach the steady state, the cumulative coefficient (Rods) based on the area under the curve (AUC), the clearance half-life in the terminal excretion phase ($t_{1/2\lambda_z}$) and comparison of AUC_{0-168h} (PEG-IFN alpha-2b) or AUC_{0-12h} for the first and final administrations were also determined. One patient whose IFN concentration exceeded the upper limit of the therapeutic range was excluded from this analysis.

Final virological response and hepatitis C virus dynamics in serum

Patients who were HCV-RNA negative at week 24 following completion of treatment were defined as having achieved an SVR. Patients who did not achieve an SVR were classified as nonresponders (NRs).

To analyse the effect of treatment on HCV dynamics, the amount of HCV-RNA was quantified at the following time points: immediately before initiation of the therapy and 4, 8, 24 h and 2, 4, 7, 14, 21, 28, 56, 84, 112, 140, 168 and 336 days after initiating therapy. The total RNA was extracted from the serum, and the amount of HCV-RNA at each time point was quantified by real-time detection PCR as reported previously [7,10]. The detection sensitivity of this assay was approximately 10 copies/mL, and the dynamic range for the method was from 10 to 1×10^8 copies/mL [11]. The viral decline curve was plotted on a semilogarithmic graph, and the slope of the exponential viral decline was calculated individually by a straight-line fit to the data for each viral decline phase.

Statistical analysis

Categorical data were compared by the chi-square test or Fisher's exact test. Distributions of continuous variables in the two treatment groups were analysed by Student's *t*-test. All tests of the confidence interval were two tailed, with the level of confidence level being set at 95%. *P*-values of <0.05 were considered statistically significant.

In order to analyse the pharmacokinetics of PEG-IFN alpha-2b and ribavirin, descriptive statistics were calculated at each blood collection, and the relationship between the time point of blood collection and the measured levels of the two drugs displayed graphically for each subject. These graphs included the mean value, standard error and the measured concentrations of the drugs at the first and after the final administration. In addition, these analyses were used to confirm the circadian trough values and to estimate the time to reach the steady state, based on AUC (Rods) and clearance half-life ($t_{1/2\lambda_z}$).

RESULTS

The demographics of the patients are shown in Table 1. No significant differences were found in mean age, gender proportionality, activity and stage of liver histology, serum ALT level and initial viral load between the PEG-IFN alpha-2b and non-PEG-IFN alpha-2b treatment groups. SVR rates in the PEG-IFN alpha-2b and non-PEG-IFN alpha-2b treatment groups were 69% (18/26) and 31% (8/26), respectively.

Differences in PKR mRNA expression in response to the different interferon treatment regimens

Sequential transcript analysis demonstrated an approximately 15-fold increase in PKR mRNA expression within 4 h following administration of conventional IFN alpha-2b. At

Table 1 Clinical characteristics of the patients in the two treatment groups of the study

	PEG-IFN alpha-2b plus ribavirin	IFN alpha-2b plus ribavirin	*P*-value (95% CI)
No. of patients	26	26	
Age (years), median (range)	53 (29–67)	53 (29–70)	0.66 (−4.18–6.57)*
Gender (male/female)	14/12	13/13	0.78†
Histology of the liver			
A1/A2/A3	12/11/3	14/11/1	0.56†
F1/F2/F3	14/10/2	13/7/6	0.28†
ALT (IU/L)	93 (72–113)	84 (63–105)	0.54 (−38.2–20.2)*
Haemoglobin (g/dL)	14.6 (14.2–15.0)	14.2 (13.6–14.9)	0.26 (−1.11–0.31)*
Platelet count ($\times 10^3$/mL)	179 (164–195)	171 (151–190)	0.47 (−3.32–1.56)*
Viral load ($\times 10^6$ copies/mL)	14.6 (9.00–20.2)	8.35 (3.77–12.9)	0.11 (−14.1–1.55)*
Ribavirin concentration at 4 W (ng/mL)	2413 (1451–3376)	2266 (1568–2963)	0.79 (−1281–985)*

Values are expressed as mean (95% CI).
*Unpaired *t*-test. †Chi-square test.

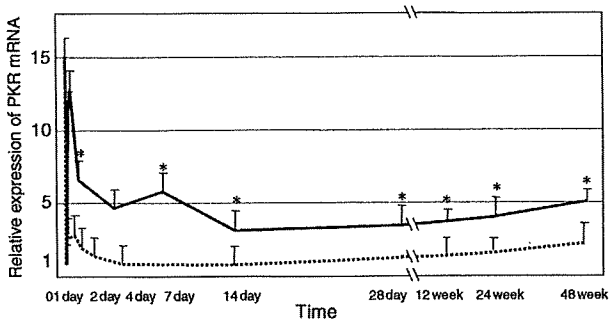

Fig. 1 Sequential expression of PKR mRNA in PBMCs during PEG- (solid line) and conventional (dotted line) IFN alpha-2b and ribavirin combination therapy. Expression of mRNA is shown as the expression level relative to baseline expression. The error bars indicate the standard error. An asterisk indicates a statistically significant difference in relative expression values between the two different IFN regimens ($P < 0.05$).

8 h, the level of PKR mRNA had fallen to a level that was twofold greater than the pre-treatment level (Fig. 1). With PEG-IFN alpha-2b administration, PKR mRNA expression reached a peak at 8 h at a level 12-fold greater than the pre-treatment level. At 24 h post-administration, the level of PKR mRNA had fallen but was still sixfold greater than the pre-treatment level (Fig. 1). This level was maintained until the next dose. No significant difference was observed in peak PKR mRNA expression between conventional IFN alpha-2b and PEG-IFN alpha-2b. However, from the second day of administration onwards, the expression was maintained at a significantly higher level in the PEG-IFN alpha-2b group compared with the conventional IFN alpha-2b group ($P < 0.05$) (Fig. 1).

Pharmacokinetics of serum pegylated interferon alpha-2b

The pharmacokinetic parameters for PEG-IFN alpha-2b at weeks 1 (first administration) and 48 (final administration) are shown in Table 2. Although the trough value of serum PEG-IFN alpha-2b varied between individuals, it almost reached a plateau at week 8. Accumulation of IFN was minimal in the PEG-IFN alpha-2b treatment regimen.

The level of serum PEG-IFN alpha-2b at week 1 increased gradually up to 12–24 h with a $t_{1/2\lambda_z}$ of 40.2 h. These levels were measurable up to 168 h after administration or immediately before the next administration. The trough value following administration showed no significant increase during the 48-week treatment phase (Fig. 2). The blood level after the final administration increased gradually for 12–24 h, remained high for approximately 48 h, and then decreased slowly with a $t_{1/2\lambda_z}$ of 55.3 h. The drug remained measurable up to 2 weeks post-administration. The cumulative coefficients (Rods) of repeated administrations calculated on the basis of C_{max}, C_{168h} and AUC_{0-168h} were 0.917, 2.11 and 1.12, respectively. When a comparison was made between the first and final administrations (weeks 1 and 48), $t_{1/2}$ of serum PEG-IFN alpha-2b levels was slightly prolonged after the final administration, although no changes were observed in C_{max}, AUC and plasma clearance (CL/F) (Table 2; Fig. 3).

Pharmacokinetics of serum ribavirin

The pharmacokinetic parameters for ribavirin at weeks 1 (first administration) and 48 (final administration) are summarized in Table 3. The trough value of serum ribavirin almost reached a plateau 8 weeks after the initial administration. In contrast to PEG-IFN alpha-2b, ribavirin was accumulated significantly during the first 4–8 weeks.

Serum ribavirin levels after the first administration (first day) reached t_{max} by 3.33 h and then decreased rapidly with a $t_{1/2\lambda_z}$ of 27.1 h. In contrast, serum ribavirin levels reached t_{max} by 2.73 h after the final administration and then decreased slowly with a $t_{1/2\lambda_z}$ of 296 h. A comparison of the cumulative coefficient (Rods) in the steady state was made between the first and final administrations and was calculated on the basis of C_{max}, C_{12h} and AUC_{0-12h}. This showed that by the final administration, there was a marked increase in C_{max} and AUC in serum ribavirin levels, an approximately 10-fold prolongation of $t_{1/2\lambda_z}$, a decrease in CL/F of about 1/3, and an approximately threefold increase in Vz/F. There was no change evident in t_{max} (Table 3; Fig. 4).

Clinical and virological response and serum pegylated interferon alpha-2b and ribavirin levels

The dose of PEG-IFN alpha-2b was reduced in two patients after 4 and 25 weeks of treatment because of neutropoenia. Similarly, the dose of ribavirin was reduced in three patients

Table 2 Pharmacokinetic parameters of the patients who received PEG-IFN alpha-2b at weeks 1 (first administration) and 48 (final administration)

	t_{max} (h)	C_{max} (pg/mL)	C_{168h} (pg/mL)	$t_{1/2\lambda_z}$ (h)	AUC (pg h/mL) 0–168 h	CL/F (mL/h/kg)	Vz/F (L/kg)
First	23.1	874	99	40.2	68 926	21.4	1.18
Final	22.2	774	185	55.3	77 039	–	–
Rods	–	0.917	2.11	–	1.12	–	–

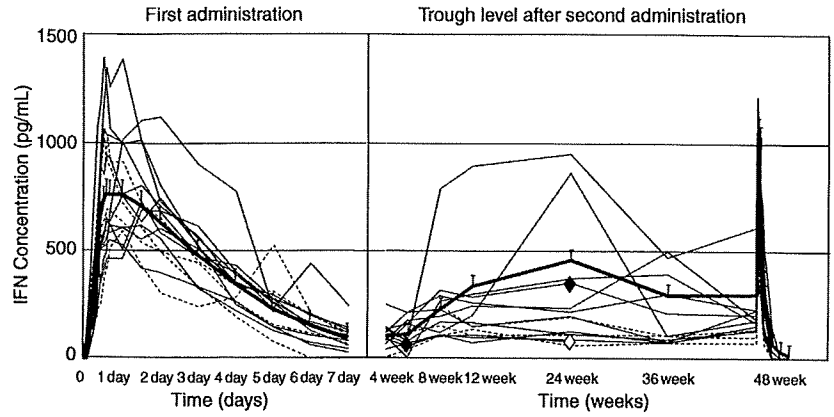

Fig. 2 Changes in serum IFN levels during PEG-IFN alpha-2b and ribavirin combination therapy. No significant increase in the trough value of serum IFN level was found during the 48-week treatment period. The bold lines indicate mean values, while the error bars indicate the standard error. Fine solid lines indicate a sustained virological responder and broken lines a nonresponder. The diamond-shaped symbol indicates a time point and IFN concentration at which either dose reduction (closed diamonds) or discontinuation (open diamonds) occurred.

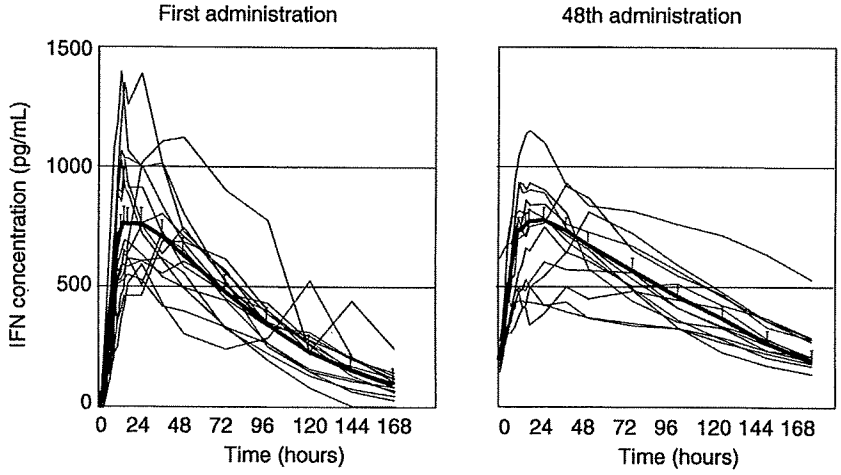

Fig. 3 A comparison of serum IFN levels between the first and 48th doses. Both show very similar values and no accumulation of IFN. It should be noted that PEG-IFN alpha-2b was detectable in all but one patient at 168 h after initial administration. Bold lines indicate mean values and the error bars indicate the standard error.

Table 3 Pharmacokinetic parameters of the patients who received ribavirin at weeks 1 (first administration) and 48 (final administration)

	t_{max} (h)	C_{max} (pg/mL)	C_{168h} (pg/mL)	$t_{1/2\lambda_z}$ (h)	AUC(pg h/mL) 0–168 h	CL/F (mL/h/kg)	Vz/F (L/kg)
First	3.33	604	221	27.1	4019	37.8	1472
Final	2.73	3449	2422	296	33 060	12.7	5374
Rods	–	6.53	12.2	–	9.42	–	–

after 12 and 16 weeks of treatment because of anaemia. In Figs 2 & 4, the individual time points and drug concentration following dose reduction are indicated by closed diamonds. No association could be found between dose reduction and serum concentration for both agents. Treatment was discontinued in 1 of the 15 patients because of depression, as indicated by open diamonds in Figs 2 & 4. Eleven patients including this patient achieved an SVR, with the remaining four patients being classified as NRs.

In order to demonstrate the association between virological response and pharmacokinetics, the final virological response for each individual is indicated in Figs 2 & 4. Serum IFN levels at 2 weeks post-dose tended to be slightly higher in NRs when compared with patients who achieved an SVR. This difference was not statistically significant. There was also no significant difference in serum ribavirin levels between these two groups from the time of the first administration until the completion of the 48-week treatment period.

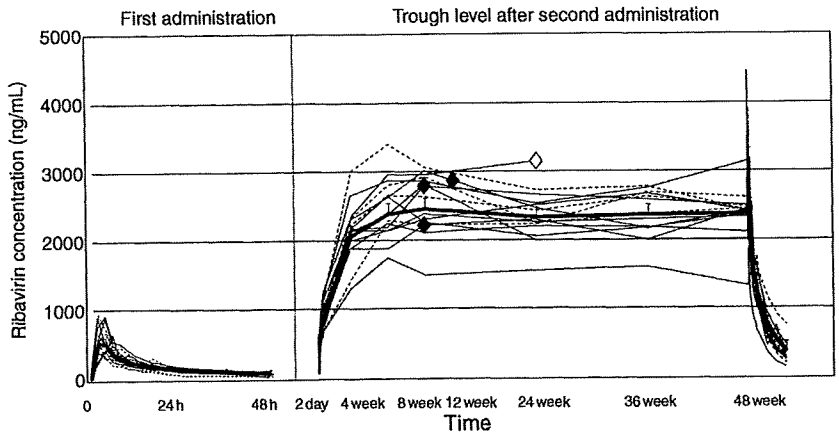

Fig. 4 Changes in serum ribavirin levels during PEG-IFN alpha-2b and ribavirin combination therapy. Serum ribavirin levels reached a peak by the eighth week and then plateaued. Bold lines indicate mean values and the error bars indicate the standard error. Fine solid lines indicate a sustained virological responder and broken lines a nonresponder. The diamond-shaped symbols indicate a time point and ribavirin concentration at which either dose reduction (closed diamonds) or discontinuation (open diamonds) occurred.

Association between PKR mRNA expression and virological response

The absolute expression levels of PKR mRNA at baseline prior to treatment were slightly higher in NRs than in SVR patients (1.8×10^{-2} *vs* 1.3×10^{-2} copies/one copy of G3PDH), although this difference was not statistically significant. Interestingly, in the PEG-IFN alpha-2b group, sequential PKR mRNA expression in response to PEG-IFN administration was significantly higher in patients who achieved an SVR compared with patients classified as NRs ($P < 0.05$) (Fig. 5).

The serum HCV dynamics during PEG-IFN alpha-2b and ribavirin combination therapy showed a biphasic pattern consisting of a rapid decrease within 24 h of initiation of the treatment (first phase), followed by a subsequent slow decrease. The mean viral decay during the first phase was 3.0 $\log_{10}$/day (95% CI: 2.4–3.5) and that calculated from day 2 onwards (the second phase of the response) was 0.075 (95% CI: 0.028–0.12) $\log_{10}$/day. Significant correlation was found between PKR expression at day 1 and viral decline rate calculated from the first phase of HCV dynamics ($r = 0.67$, $P = 0.0006$) (Fig. 6a). Moreover, significant correlation was also found between PKR expression at day 84 and second phase viral decline rate ($r = 0.67$, $P = 0.001$) (Fig. 6b). No significant associations were found between PEG-IFN or ribavirin concentration and kinetics of PKR expression.

DISCUSSION

The data of this study suggests that the higher expression levels of PKR transcripts seen with PEG-IFN alpha-2b from the second day of administration onwards were related, at least in part, to the improved efficacy of PEG-IFN alpha-2b compared with conventional IFN alpha-2b. Our pharmacokinetic study suggests that pegylation may be responsible for the dramatic effect on induction of PKR associated with the PEG-IFN regimen, possibly as a consequence of maintaining blood levels of IFN within the therapeutic range. This concept is supported by our previous work [8], in which we demonstrated that intracellular expression of PKR during the second phase was maintained at a significantly higher level when IFN-beta was administered twice daily.

The expression of PKR transcripts was induced very rapidly following the first administration, and PKR expression at day 1 was significantly correlated with the first phase viral decline rate of HCV dynamics. It is likely this increase in PKR transcripts was associated with the rapid decline of HCV seen in the first phase of serum HCV dynamics, and this change is believed to be a result of the direct effect of IFN on virion production and release from infected target cells [12]. Although we found that there was no significant difference in peak PKR mRNA expression between the PEG-IFN alpha-2b and IFN alpha-2b groups, the expression of PKR transcripts from 24 h onwards was significantly higher with PEG-IFN alpha-2b than conventional IFN alpha-2b administration. The decline in viral numbers and activity seen after the second day (second phase viral decline of HCV dynamics) is believed to reflect the presumed elimination of viral-infected cells in addition to the direct antiviral properties of IFN [12]. It has been suggested recently that apoptosis of HCV-infected cells induced by IFN-stimulated PKR may be an important mechanism for the elimination of viruses [13]. In the present study, expression of PKR transcripts in response to PEG-IFN administration was higher in patients who achieved SVR compared with NR patients, and expression of PKR at day 84 was significantly associated with the viral decline rate calculated from the second phase of HCV dynamics. Therefore, the increased expression of PKR transcripts we observed

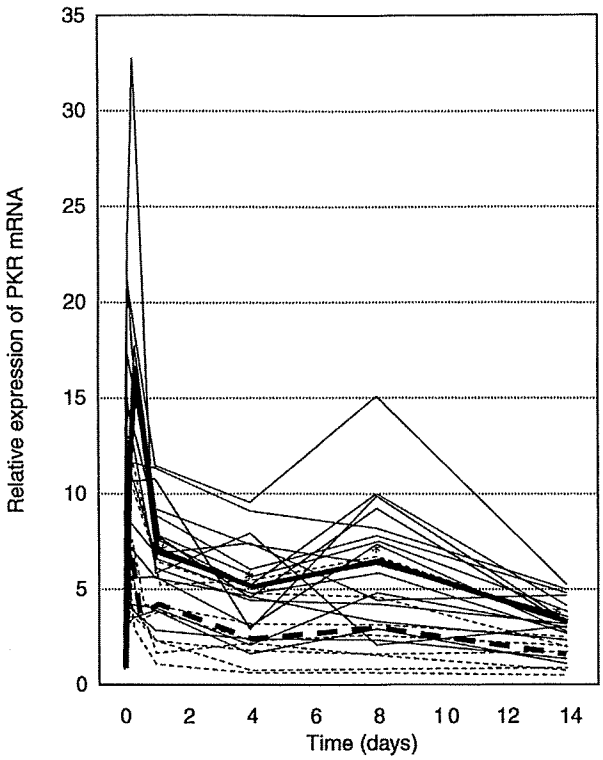

Fig. 5 Sequential expression of PKR mRNA in PBMCs in sustained viral responders (solid line, $n = 18$) and nonresponders (dotted line, $n = 8$). The bold line indicates the mean value for each group. Expression of mRNA is shown as the expression level relative to baseline expression. An asterisk indicates a statistically significant difference in relative expression value between the two different virological responses ($P < 0.05$).

after the second day may be associated with the enhanced efficacy of PEG-IFN alpha-2b. Again, this increased expression may have been due to an improvement in the pharmacokinetics of IFN following pegylation that results in prolonged clearance of IFN from serum.

Gerotto et al. [14] reported previously that higher baseline PKR expression was observed in NR patients compared with patients who achieved an SVR, although no significant difference was found in 'absolute' expression of PKR during treatment between these patients. We observed a similar trend in baseline expression in our study, although the relatively small number of cases meant that this difference did not achieve statistical significance. However, in our study, increased expression of PKR in response to PEG-IFN treatment was found in patients with an SVR. We analysed the changes in PKR expression during treatment relative to baseline expression levels. Because the absolute expression of PKR in response to IFN varies between patients (data not shown), we believe that calculating the level of expression during IFN treatment relative to the level of baseline expression is suitable in comparing PKR responses between patients. While this issue still remains controversial, our results imply that no or low responsiveness of PKR (i.e. less than a twofold increase from baseline) is associated negatively with an SVR, although high responsiveness of PKR during PEG-IFN administration does not always assure an SVR.

Although PBMCs were used as a model to quantify the serial gene expression of PKR, expression of PKR should be studied with hepatocytes, the target cell of HCV. Using liver tissue for sequential analysis is more ideal but ethically impossible. To address this point, we previously demonstrated a significant correlation between basal expression of PKR in liver tissue and the corresponding PBMC [8].

One of the limitation of the present study is that our results specifically concern PKR. Therefore, our present findings cannot be extrapolated to other ISGs such as MxA and 2′,5′-oligoadenylate synthetase. Although expression and response of ISGs to therapy may differ among different ISGs, we previously found significant correlation between sequential expression levels for PKR and MxA during IFN treatment [8].

In the present study, PEG-IFN alpha-2b was detectable in all but one patient at 168 h after initial administration in

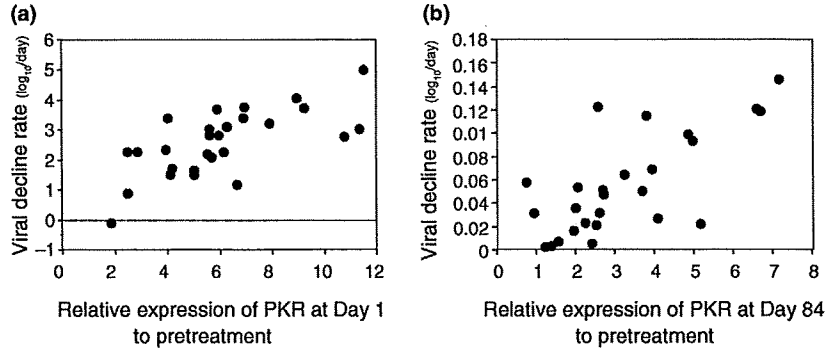

Fig. 6 (a) Significant correlation between expression of PKR mRNA at day 1 and viral decline rate calculated from the first phase of HCV dynamics ($r = 0.67$, $P = 0.0006$). (b) Significant correlation between expression of PKR mRNA at day 84 and viral decline rate calculated from the second phase of HCV dynamics ($r = 0.67$, $P = 0.001$).

contrast to a study reported by Bruno et al. [15]. However, as in that study, no significant accumulation of PEG-IFN alpha-2b was found during therapy, which is marked contrast to the data from PEG-IFN alpha-2a (40 kD) plus ribavirin therapy [15]. In our study, the viral response was not associated with serum PEG-IFN concentration, but it was associated with cellular responses to IFN such as PKR expression. Although both PEG-IFNs appear to show different profiles in absorption, distribution and clearance, it remains unknown how these differences relate to differences in cellular responses *in vivo* such as PKR and the primary clinical endpoint, SVR.

The serum level of ribavirin has been reported previously to be associated with the observed clinical effects [6]. With ribavirin combination therapy, the antiviral effect was more potent after 3 weeks, at which time serum ribavirin levels were shown to have increased [7]. Therefore, accumulation of ribavirin from the third week of administration onwards, during which viral suppression is important for SVR, may be associated with the viral response seen with combination therapy. However, in our study, we found no significant difference in serum ribavirin levels between patients who achieved an SVR and NR patients. There was also no significant difference in serum IFN levels between the SVR and NR patients. As there are only a small number of studies that have reported serum ribavirin levels and associated virological effects in detail, further more comprehensive investigations are therefore required.

In conclusion, the pharmacokinetic improvement provided by pegylation of IFN leads to dramatic changes in PKR transcript expression patterns. In contrast, serum ribavirin concentrations appear not to be associated with the viral response and PKR expression. Our data suggest that the higher intracellular expression of PKR transcripts from the second day onwards is associated with the enhanced virological efficacy of PEG-IFN alpha-2b and ribavirin combination therapy.

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The presence of steatosis and elevation of alanine aminotransferase level are associated with fibrosis progression in chronic hepatitis C with non-response to interferon therapy.

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Title: Potential relevance of cytoplasmic viral sensors and related regulators involving innate immunity in antiviral response

Short title: Innate immunity and therapeutic response

Authors: Yasuhiro Asahina, M.D.[1], Namiki Izumi, M.D.[1], Itsuko Hirayama, M.D.[1], Tomohiro Tanaka, M.D.[1], Mitsuaki Sato, M.D.[1,2], Yutaka Yasui, M.D.[1], Nobutoshi Komatsu, M.D.[1,2], Naoki Umeda, M.D.[1], Takanori Hosokawa, M.D.[1], Ken Ueda, M.D.[1], Kaoru Tsuchiya, M.D.[1], Hiroyuki Nakanishi, M.D.[1], Jun Itakura, M.D.[1], Masayuki Kurosaki, M.D.[1], Nobuyuki Enomoto, M.D.[2], Megumi Tasaka, M.D.[3], Naoya Sakamoto, M.D.[3] and Shozo Miyake, M.D.[1]

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Technology; and the Japanese Ministry of Welfare, Health and Labor.

Abbreviations: HCV, hepatitis C virus; PEG-IFN, pegylated interferon; NVR, non-virological responders; RIG-I, retinoic acid-inducible gene I; MDA5, melanoma differentiation associated gene 5; CARD, Caspase-recruiting domain; Cardif, caspase-recruiting domain adaptor inducing IFN-beta; IPS-1, IFN-beta promoter stimulator I; MAVS, mitochondrial antiviral signaling protein; VISA, virus-induced signaling adaptor; RNF125, ring-finger protein 125; ISG15, IFN-stimulated gene 15; USP18, ubiquitin-specific protease 18; UBP43, ubiquitin-specific protease 43; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; HMBS, hydroxymethylbilane synthase; PBMC, peripheral blood mononuclear cell; SVR, sustained viral responder; TR, transient responder; ROC, receiver operator characteristic; JAK, Janus kinase.

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External Validation of FIB-4: Diagnostic Accuracy Is Limited in Elderly Populations

To the Editor:

We read with interest the articles by Stering et al.[1] and Vallet-Pichard et al.[2] The former authors developed the FIB-4 index, a noninvasive method for assessing liver fibrosis in patients with HIV/HCV coinfection. The variables used are age, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and platelet (PLT) count, and the formula is as follows: (age [yr] × AST [U/L])/ ((PLT[10^9/L]) × (ALT[U/L])$^{1/2}$). They showed that over 70% of patients could be classified into either absence or presence of advanced fibrosis by cutoff of <1.45 or >3.25 respectively, with diagnostic accuracy of 87%. The latter authors expanded the applicability of the FIB-4 index to HCV-monoinfected patients and showed that 73% of patients were classified with diagnostic accuracy of 93%, an excellent performance in both classification and accuracy of diagnosis.

Because the mean age of patients was young in these studies (40 years[1] and 44 years[2]), we wondered whether this index could also fit to Japanese patients who are rather older than the Western patients. We validated the FIB-4 index in a retrospective cohort of 1,405 patients who underwent liver biopsy at our hospital. The mean age was 55 ± 12 years. The distribution of METAVIR fibrosis scores was as follows: 1.6% showed no fibrosis (F0), 44.8% showed mild fibrosis (F1), 29.5% showed moderate fibrosis (F2), 20.2% showed severe fibrosis (F3), and 3.9% showed cirrhosis (F4). The proportion of advanced fibrosis (F3 or F4) was slightly higher in our population compared to the former studies (24.1% vs. 20.7% [1] and 17.2% [2]). As shown in Table 1, only 53% of patients were classified to either <1.45 or >3.25, a much lower rate than previous reports. The diagnostic accuracy was excellent in patients with a FIB-4 index <1.45 (94%), however, it was relatively poor in patients with a FIB-4 index >3.25 (50%) making the overall accuracy as low as 67%.

We supposed this discordance with previous reports may be derived from the older age of our populations and thus we categorized patients into three groups according to age and analyzed separately. In patients with age ≤50 years, 64% of patients were classified, and the diagnostic accuracy was 94% for a FIB-4 index <1.45 and 68% for a FIB-4 index >3.25 making the overall accuracy of 90%, a result comparable to previous reports. In older patients, however, diagnostic accuracy was significantly low compared to those with age ≤50 years (56% for age 51-60 years, $P < 0.0001$ and 51% for age ≥60 years, $P < 0.0001$). Because patients with a FIB-4 index >3.25 increased according to age (6%, 34%, and 53% for ages ≤50, 51-60 and >60 years), and the diagnostic accuracy was low in these patients (48% to 50%), these results suggest that, in elderly patients, a variable "age" generates excessively high FIB-4 index leading to misclassification of no-moderate fibrosis (F0-F2) into a FIB-4 index >3.25.

In conclusion, the FIB-4 index could accurately differentiate advanced fibrosis in young Japanese patients with chronic hepatitis C but the diagnostic accuracy is limited in the elderly. Thus, in elderly patients, some sort of adjustment for the effect of age on FIB-4 index may be necessary for more precise classification.

Table 1. Comparison of FIB-4 Index and Liver Biopsy Results in Terms of Age

		METAVIR Fibrosis Score			Diagnostic Accuracy
	FIB-4	F0-2	F3-4	Total	
All patients					
	<1.45	283 (20%)	18 (1%)	301 (21%)	94%
	>3.25	228 (16%)	226 (16%)	454 (32%)	50%
	1.45-3.25	556 (40%)	94 (7%)	650 (47%)	
	Total	1067 (76%)	338 (24%)	1405 (100%)	67%
Age ≤50	<1.45	240 (54%)	16 (4%)	256 (58%)	94%
(Mean 40 yrs)	>3.25	9 (2%)	19 (4%)	28 (6%)	68%
	1.45-3.25	126 (28%)	38 (8%)	164 (36%)	
	Total	375 (84%)	73 (16%)	448 (100%)	90%
Age 51-60	<1.45	30 (7%)	2 (1%)	32 (8%)	94%
(Mean 56 yrs)	>3.25	76 (18%)	69 (16%)	145 (34%)	48%
	1.45-3.25	215 (50%)	36 (8%)	251 (58%)	
	Total	321 (75%)	107 (25%)	428 (100%)	56%
Age >60	<1.45	13 (2%)	0 (0%)	13 (2%)	100%
(Mean 66 yrs)	>3.25	143 (27%)	138 (26%)	281 (53%)	49%
	1.45-3.25	215 (41%)	20 (4%)	235 (45%)	
	Total	371 (70%)	158 (30%)	529 (100%)	51%

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Journal of Gastroenterology

Pretreatment predictor of response, time to progression, and survival to intraarterial 5-fluorouracil/interferon combination therapy in patients with advanced hepatocellular carcinoma

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Background. Several studies have reported survival benefits of combination therapy with intraarterial 5-fluorouracil (5-FU) and subcutaneous interferon (IFN) α for advanced hepatocellular carcinoma (HCC) with portal vein tumor thrombosis (PVTT). We investigated the pretreatment predictive factors of early response, time to progression (TTP), and survival in response to intraarterial 5-FU/IFN combination therapy. ***Methods.*** Patients with nonresectable HCC and variable PVTT grades (without PVTT to PVTT in the trunk) received intraarterial 5-FU/IFN combination therapy (n = 55). ***Results.*** After two courses of the combination therapy, 1 (2%), 15 (27%), 16 (29%), 12 (22%), and 11 (20%) of 55 patients showed complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), or had dropped out (DO), respectively, when their early response to treatment was assessed. Univariate analysis identified only hepatitis C virus (HCV) antibody positivity as having significantly influenced the early response (P = 0.028) and TTP (P = 0.021). Multivariate analysis identified performance status (P = 0.003) and HCV antibody positivity (P = 0.007) as significant and independent determinants of survival. PVTT grade did not influence early response, TTP, or survival. The survival rate was significantly higher in patients who achieved CR or PR than in those that assessed as SD or PD, or DO (P < 0.0001, each). ***Conclusions.*** HCV antibody positivity may be a significant pretreatment predictor of early response, TTP, and survival of patients with advanced HCC treated with 5-FU/IFN. CR or PR as the early response to the combination therapy might indicate a more favorable prognosis in patients with advanced HCC. PVTT grade did not seem to influence the efficacy of combination therapy.

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Key words: advanced hepatocellular carcinoma, 5-fluorouracil and interferon, early response, survival, HCV

Introduction

Hepatocellular carcinoma (HCC) is a life-threatening neoplasm and one of the most common neoplasms in Africa and Asia, including Japan. Deaths due to HCC are increasing worldwide.[1–3] Advances in biotechnology have resulted in new diagnostic techniques, such as ultrasonography, computed tomography (CT), magnetic resonance imaging, and angiography. Similarly, new treatment options have become available, such as surgical resection, radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), and transcatheter arterial chemoembolization (TACE). As a result, the prognosis of HCC patients has gradually improved. Nevertheless, the survival rates of patients with advanced HCC and complications such as portal vein tumor thrombosis (PVTT) or distant metastasis remains extremely poor.[4–8]

Advances in implantable drug delivery systems have allowed repeated arterial infusions of anticancer agents. First, monotherapy with intraarterial 5-fluorouracil (5-FU) for unresectable HCC was reported.[9,10] However, such treatment resulted in a low response rate (13.0% and 22.0%). Next, several authors reported favorable results with low-dose cisplatin and 5-FU for advanced HCC with PVTT, with a response rate ranging from 33.0% to 48.0%.[11–13] Recently, several studies have reported survival benefits of combination therapy with intraarterial 5-FU and subcutaneous interferon (IFN) α for advanced HCC with PVTT, with a response rate ranging from 43.6% to 72.7%.[14–17] In these studies, only HCC patients with PVTT (in the main trunk or first branch) without distant metastases were treated. The

pretreatment predictive factors of response, time to progression (TTP), and survival of HCC patients treated with the combination therapy remain unclear. At present, some patients with nonresectable HCC are treated with TACE. However, some patients are not suitable candidates for TACE because of PVTT or poor response to TACE. Because of the poor prognosis of patients with nonresectable HCC who are not treatable by TACE, effective treatment is needed. There is little information about assessment of patients with advanced HCC (e.g., nonresectable HCC with PVTT in the second branch or nonresectable HCC without PVTT but with poor response to TACE) treated with combination therapy of intraarterial 5-FU and IFN. In the present retrospective cohort study, we assessed the efficacy of intraarterial 5-FU with IFN for various types of nonresectable advanced HCC and investigated the pretreatment predictive factors of early response, TTP, and survival in response to the combination therapy.

Materials and methods

Patients

From June 2003 to December 2006, 265 consecutive patients with unresectable HCC were admitted to our hospital. Of the 265 patients with advanced HCC, 94 were treated with TACE, 34 patients received systemic chemotherapy, and 13 patients received best supportive care. The remaining 124 patients were selected as suitable candidates for intraarterial 5-FU and IFN combination therapy. Forty-one patients refused the therapy. Thus, 83 patients with advanced HCC were treated with intraarterial 5-FU and IFN. Of these 83 patients, 24 with distant metastases and four with hepatic venous invasion were excluded from this study, so we assessed 55 patients without distant metastases or hepatic venous invasion in this retrospective cohort study. Of the 55 patients, 30 had been treated with TACE before enrollment. Table 1 lists the baseline characteristics of the 55 patients. PVTT grade, based on the location of the tumor thrombus, was determined according to the criteria of the Liver Cancer Study Group of Japan (LCSGJ).[18] PVTT grading was as follows: Vp 0, no PVTT; Vp 1, tumor thrombus in a third or more of the peripheral branches of the portal vein; Vp 2, tumor thrombus in a second branch of the portal vein; Vp 3, tumor thrombus in the first branch of the portal vein; and Vp 4, tumor thrombus in the trunk of the portal vein. Tumor staging was defined based on the TNM staging system of the LCSGJ:[18] stage I (fulfilling three intrahepatic conditions: solitary, <2 cm, no vessel invasion), stage II (fulfilling two of the three intrahepatic conditions), stage III (fulfilling one of the three intrahepatic conditions), stage IVA (fulfilling none of the three intrahepatic conditions with no distant metastases or any intrahepatic conditions with lymph node metastases), and stage IVB (any intrahepatic conditions with distant metastases).

Eligibility

This was a retrospective cohort study to investigate pretreatment predictive factors of TTP, survival, and

Table 1. Clinical profile of the 55 HCC patients

Age (years)[a]	67 (38–79)
Sex (M/F)	44/11
Etiology: HBV/HCV/other	15/36/4
Total bilirubin (mg/dl)	1.1 (0.4–6.4)
Platelet count ($\times 10^4$ mg/dl)	13.0 (5.1–54.5)
Albumin (mg/dl)	3.5 (2.4–4.8)
Child Pugh stage (A/B/C)	43/10/2
PS (0/1)	45/10
Intrahepatic tumor volume (≤50%/>50%)	38/17
Tumor stage (III/IVA)	20/35
Vp[a] (0/2/3/4)	20/6/15/14
AFP (ng/ml)	934 (14.3–525 900)
AFP-L3 (%)	47.3 (<0.5–87.6)
DCP (mAU/ml)	3729 (10–722 140)
Previous treatment (performed/not performed)	30/25

Data are expressed as median with range values in parentheses, or number of patients HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus; PS, Eastern Cooperative Oncology Group performance status; AFP, α-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of α-fetoprotein; DCP, des-γ-carboxy prothrombin; PVTT, portal vein tumor thrombosis

[a] PVTT grade: Vp 0, no PVTT; Vp 1, tumor thrombus in a third or more of the peripheral branches of the portal vein; Vp 2, tumor thrombus in a second branch of the portal vein; Vp 3, tumor thrombus in the first branch of the portal vein; Vp 4, tumor thrombus in the trunk of the portal vein

response to intraarterial 5-FU/IFN combination therapy. Eligibility criteria were as follows: age, 18–80 years; leukocyte count, >2000/μl; neutrophil count, >1200/μl; hemoglobin, >8 g/dl; platelet count, >50000/μl; unresectable or not suitable for local ablation therapy, including RFA or PEI; with PVTT or TACE was ineffective; without hepatic venous invasion; without distant metastases; and Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1.[19] There was no eligibility criterion regarding hepatic reserve function, including serum total bilirubin levels. All patients gave written informed consent to this study, which was approved by the Institutional Review Board of Hiroshima University.

Treatment protocol

Patients received repeated arterial infusions of anticancer agents via the injection port. One course of chemotherapy lasted 4 weeks. 5-FU (500mg body weight/day, Kyowa Hakko, Tokyo, Japan) was administered over 5h with a mechanical infusion pump on days 1 to 5 of the first and second weeks (5g in one course). Recombinant IFN α-2b (Intron A, Schering-Plough Pharmaceuticals, Osaka, Japan); 3×10^6U (3MU), or natural IFN α (OIF, Otsuka Pharmaceuticals, Tokyo, Japan); 5×10^6U (5MU) was administered intramuscularly on days 1, 3, and 5 of each week (total dose, 36 and 60MU, respectively). In principle, treatment was repeated several times unless PS changed to 3 or 4 during the treatment. A 2- to 4-week rest period of no treatment was allowed after each treatment course. As for the two types of IFN, we previously reported similar effects of recombinant IFN α-2b and natural IFN α when combined with intraarterial 5-FU for the treatment of advanced HCC.[20]

Implantation of the arterial catheter

A catheter was inserted through the right femoral artery by the Seldinger method. After localization of the HCC, a 3-French heparin-coated catheter was inserted and its tip advanced to the common hepatic artery or proper hepatic artery. The other end of the catheter was connected to the injection port, which was implanted in a subcutaneous pocket created in the right lower abdominal quadrant. The gastroduodenal artery and right gastric artery were occluded with steel coils to prevent gastroduodenal injury by the chemotherapeutic agents.

Evaluation

The early response to the combination therapy was assessed with contrast-enhanced CT after two courses of the combination therapy. The response was defined according to the criteria of the Response Evaluation Criteria in Solid Tumors (RECIST).[21] A complete response (CR) was defined as the complete disappearance of all target lesions. A partial response (PR) was defined as a decrease of at least 30% in the sum of the longest diameter of the target lesions with the baseline sum of the longest diameter of the target lesions as the reference. Progressive disease (PD) was defined as an increase of at least 20% in the sum of the longest diameter of target lesions. Stable disease (SD) was defined as meeting neither the PR nor the PD criteria. The duration of the response was measured from the date of the start of treatment to the date of documented progression. Adverse reactions were assessed with the National Cancer Institute Common Toxicity Criteria (NCI-CTC; version 3.0)[22] every week during the treatment.

Additional therapy

After two courses of the combination therapy, we assessed the response to therapy in all patients. According to the response, we provided various additional therapies such as RFA, TACE, or radiotherapy (RT) to patients treated with the combination therapy. These additional therapies were considered for patients with PS of 0–1 and a Child-Pugh stage of A or B. Patients assessed with PR continued to receive the combination therapy repeatedly. Then, when downstaging of advanced HCC was achieved (single tumor ≤50mm in diameter or 1–3 tumors ≤30mm in diameter) by the repeated combination therapy, RFA was considered. For patients assessed with SD or PD, in addition to the combination therapy, TACE with cisplatin–lipiodol suspension was performed. The catheter tip was advanced superselectively into the feeding artery so that sufficient anticancer agent was delivered. Among the patients assessed with SD or PD, RT was performed for PVTT if present. For patients assessed with CR, the clinical course was observed without adjuvant chemotherapy or additional therapy.

Statistical analysis

Statistical analysis was performed on 1 April 2007. Differences between groups were examined for statistical significance using the Mann-Whitney *U* test, logistic regression test, or χ-squared test as appropriate. Cumulative survival rate and TTP were calculated from the initial date of the combination therapy and assessed by the Kaplan-Meier life-table method, and differences were evaluated by the log rank test. Univariate and multivariate analyses of predictors for early response to the combination therapy were assessed by logistic

regression test. Univariate analysis of predictors of TTP and survival of patients with HCC who received the combination therapy was assessed by the Kaplan-Meier life-table method, and differences were evaluated by the log rank test. Multivariate analysis of predictors of TTP and survival was assessed by Cox proportional hazard model. Statistical significance was defined as a P value of less than 0.05. All analyses described above were performed with SPSS software (version 11, SPSS, Chicago, IL, USA). In this study, we investigated pretreatment predictive factors of early response, TTP, and survival in response to the combination therapy.

Results

Response to the combination therapy

The early response of the 55 patients was assessed after two courses of 5-FU/IFN combination therapy. As a result, 1 (2%), 15 (27%), 16 (29%), 12 (22%), and 11 (20%) patients showed CR, PR, SD, PD, or dropped out (DO), respectively. The reasons for DO were confusion (one patient), refusal after initiation of therapy (one patient), exanthema (one patient), infection around the catheter (four patients), and stenosis of the hepatic artery (four patients). We investigated the pretreatment determinants of the early response to the combination therapy. Univariate analysis identified positivity to HCV antibody as the only factor with significant influence on the early response (P = 0.028, Table 2, Fig. 1). Of the HCV antibody-positive patients, 38.9% (14/36) showed an early response of CR or PR, but only 10.5% (2/19) of other patients. When we compared the early response between patients with Vp 0–2 and those with Vp 3/4, 30.8% (8/26) of patients with Vp 0–2 and 27.6% (8/29) of those with Vp 3/4 achieved CR or PR, but the difference was not significant.

Time to progression

The median TTP in all 55 patients was 7.5 months [95% confidence interval (CI), 5.1–9.9 months], and the cumulative TTP rates at 6, 12, 18, and 24 months were 60%, 41%, 30%, and 24%, respectively. We investigated the pretreatment determinants of TTP after initiation of the combination therapy. Univariate analysis identified positivity for HCV antibody as the only factor with significant influence on TTP (P = 0.021, Table 3, Fig. 2). The median TTP in patients with Vp 0–2 and those with Vp 3/4 was 5.2 and 7.5 months, respectively. There was no significant difference in TTP between these two groups.

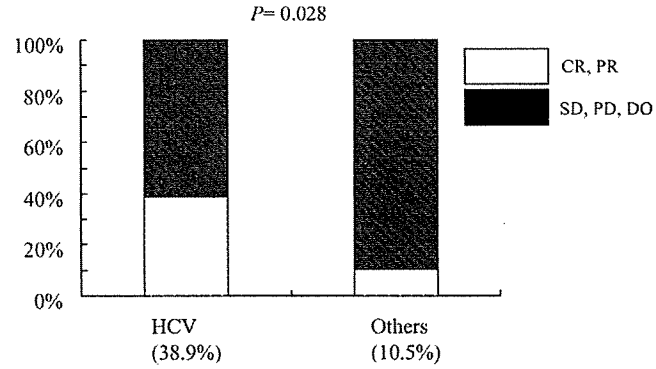

Fig. 1. Comparison of the early response rate between the hepatitis C virus (HCV)-positive group and others. The rate was significantly higher in the HCV-positive group (logistic regression test: P = 0.028). *CR*, complete response; *PR*, partial response; *SD*, stable disease; *PD*, progressive disease; *DO*, dropped out

Table 2. Univariate analysis of predictors for early response to 5-FU/IFN combination therapy

Variable	Odds Ratio	95% CI	P value
Age (≤65 vs. >65 years)	0.463	0.136–1.572	0.217
Sex (M vs. F)	2.327	0.445–12.168	0.317
HCV antibody (positive vs. negative)	6.071	1.216–30.314	0.028
Total bilirubin (≤1.5 vs. >1.5 mg/dl)	0.931	0.240–3.614	0.918
Platelet count (≤150000 vs. >150000 mg/dl)	0.978	0.278–3.437	0.972
Albumin (≤3.5 vs. >3.5 mg/dl)	1.390	0.441–4.376	0.574
Child Pugh stage (A vs. B, C)	2.172	0.413–11.420	0.360
PS (0 vs. 1)	4.965	0.576–42.810	0.145
Intrahepatic tumor volume (≤50% vs. >50%)	1.690	0.458–6.237	0.431
Tumor stage (III vs. IVA)	1.709	0.533–5.478	0.367
Vp (0–2 vs. 3, 4)	0.988	0.314–3.106	0.983
AFP (≤10000 vs. >10000 ng/ml)	0.978	0.278–3.437	0.972
AFP-L3 (≤50 vs. >50%)	0.776	0.229–2.625	0.683
DCP (≤10000 vs. >10000 mAU/ml)	0.606	0.186–1.974	0.406
Treatment (performed vs. not performed)	1.833	0.563–5.970	0.314

5-FU, 5-fluorouracil; IFN, interferon; CI, confidence interval

Table 3. Univariate analysis of predictors of time to progression

Variable	Hazard Ratio	95% CI	*P* value
Age (>65 vs. ≤65 years)	1.348	0.177–10.263	0.773
Sex (M vs. F)	1.788	0.403–7.935	0.445
HCV antibody (positive vs. negative)	2.775	1.169–6.590	0.021
Total bilirubin (≤1.5 vs. >1.5 mg/dl)	0.618	0.216–1.768	0.370
Platelet count (≤150000 vs. >150000 mg/dl)	0.739	0.307–1.777	0.500
Albumin (≤3.5 vs. >3.5 mg/dl)	0.705	0.300–1.655	0.421
Child Pugh stage (A vs. B, C)	2.381	0.314–18.045	0.401
PS (0 vs. 1)	1.348	0.177–10.263	0.773
Intrahepatic tumor volume (≤50% vs. >50%)	0.710	0.298–1.691	0.440
Tumor stage (III vs. IVA)	1.107	0.469–2.616	0.816
Vp (0–2 vs. 3, 4)	1.195	0.512–2.790	0.680
AFP (≤10000 vs. >10000 ng/ml)	1.325	0.484–3.626	0.584
AFP-L3 (≤50% vs. >50%)	2.371	0.696–8.076	0.167
DCP (≤10000 vs. >10000 mAU/ml)	1.145	0.486–2.701	0.756
Treatment (performed vs. not performed)	0.671	0.282–1.595	0.367

Table 4. Univariate analysis of predictors of survival of patients with HCC who received 5-FU/IFN combination therapy

Variable	Hazard Ratio	95% CI	*P* value
Age (≤65 vs. >65 years)	0.763	0.402–1.449	0.408
Sex (M vs. F)	1.208	0.527–2.769	0.655
HCV antibody (positive vs. negative)	2.283	1.165–4.474	0.016
Total bilirubin (≤1.5 vs. >1.5 mg/dl)	0.628	0.308–1.278	0.199
Platelet count (≤150000 vs. >150000 mg/dl)	0.690	0.355–1.340	0.273
Albumin (≤3.5 vs. >3.5 mg/dl)	0.760	0.398–1.451	0.406
Child Pugh stage (A vs. B, C)	0.527	0.228–1.216	0.133
PS (0 vs. 1)	3.413	1.391–8.375	0.007
Intrahepatic tumor volume (≤50% vs. >50%)	0.753	0.383–1.481	0.411
Tumor stage (III vs. IVA)	0.670	0.342–1.313	0.243
Vp (0–2 vs. 3, 4)	0.745	0.389–1.427	0.374
AFP (≤10000 vs. >10000 ng/ml)	0.947	0.445–2.017	0.888
AFP-L3 (≤50% vs. >50%)	0.898	0.430–1.871	0.773
DCP (≤10000 vs. >10000 mAU/ml)	0.753	0.394–1.438	0.390
Treatment (performed vs. not performed)	0.627	0.319–1.230	0.175
Additional therapy (performed vs. not performed)	1.129	0.583–2.188	0.719

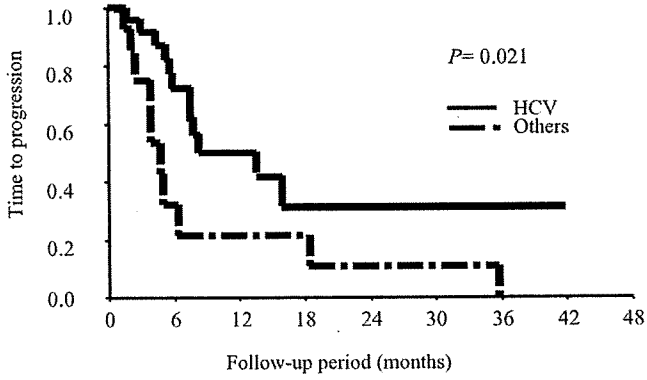

Fig. 2. Comparison of the time to progression between the HCV antibody-positive group and others. The rate was significantly higher in the HCV-positive group (log-rank test: $P = 0.021$)

Survival

The median survival in the whole group was 9.0 months (95% CI, 7.0–11.0 months), and the cumulative survival rates at 6, 12, 18, and 24 months were 67%, 39%, 22%, and 17%, respectively. We investigated the pretreatment determinants of survival after initiation of the 5-FU/IFN combination therapy. Univariate analysis identified PS = 0 ($P = 0.007$) and positivity for HCV antibody ($P = 0.016$) (Table 4, Fig. 3) as factors that significantly influenced survival. Since it was possible that the variables were mutually correlated, we performed a multivariate analysis and identified PS = 0 ($P = 0.003$) and positivity for HCV antibody ($P = 0.007$) as significant and independent determinants of survival (Table 5). The median survival time of patients with Vp 0–2 and of those with Vp 3/4 was 13.0 and 8.0 months, respectively. There was no significant difference in survival between these two groups.