CLINICAL GASTROENTEROLOGY AND HEPATOLOGY 2005;3:S89–S91

Natural Course of Asymptomatic Hepatitis C Virus–Infected Patients and Hepatocellular Carcinoma After Interferon Therapy

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A long-term follow-up study was performed to identify the natural course of chronic HCV carriers with persistently normal serum ALT level (PNAL; ≤30 U/L) and to clarify the effect of interferon therapy on the inhibition of the development of hepatocellular carcinoma (HCC) in chronic hepatitis C patients with elevated ALT levels. One hundred twenty-nine HCV carriers with PNAL underwent liver biopsy, 69 were followed for more than 5 years, and 35 underwent serial liver biopsies. We included 1246 chronic hepatitis C patients (stage F1: 231, F2: 638, F3: 336, F4: 41) who received interferon therapy and were followed for more than 2 years (mean, 7.7 years). Approximately 90% of HCV carriers with PNAL had normal to mild liver histology, and 30% developed symptomatic chronic hepatitis C within 5 years. The frequency of steatosis and iron loading was significantly lower in these patients than in symptomatic chronic hepatitis C patients. The progression rate of fibrosis was slower than in chronic hepatitis C patients with elevated serum ALT levels. HCC was noted in 157 chronic hepatitis C patients after interferon therapy, and the development of HCC was significantly reduced in both sustained responders and transient biochemical responders compared with nonresponders. HCC in sustained responders mainly developed in male patients older than 55 years with advanced stage liver histology at entry. Approximately 30% of HCV-infected patients with PNAL become candidates for antiviral therapy within 5 years. Interferon therapy lowers the rate of the development of HCC in both sustained responders and transient biochemical responders.

Hepatocellular carcinoma (HCC) occurs at a high rate in the patients with advanced stage chronic hepatitis C,[1–3] and 20%–30% of HCV-infected patients change to patients with persistently normal serum ALT levels (PNAL; ≤40 U/L).[4,5] These asymptomatic HCV carriers are predominantly female. There are a few reports that HCV carriers with PNAL showed slower progression of liver fibrosis[6–8] compared with patients with chronic hepatitis C with elevated serum ALT levels; however, Puoti et al[9] reported that HCV-infected patients with normal serum ALT (≤40 U/L) had a more progressed stage of liver fibrosis. This discrepancy might be mainly derived from the difference in the definition of asymptomatic HCV carriers and the range of normal serum ALT in each institution. Recently, an Italian group[10] demonstrated that normal range of serum ALT level was ≤30 U/L in men and ≤19 U/L in women.

Thus, it is important to clarify the pathophysiology and natural course of HCV carriers with PNAL and to clarify whether they are candidates for antiviral treatment. We also aimed to clarify the effect of interferon (IFN) therapy on the development of HCC in chronic hepatitis C patients in each stage of hepatic fibrosis including sustained responders (SRs) to IFN therapy.

Materials and Methods

HCV carriers with PNAL were defined as follows: serum ALT ≤30 U/L more than 1 year and at least 3 different occasions and platelet count of more than 150,000/μL. Patients with diabetes mellitus and obesity (body mass index, more than 30 kg/m^2) were excluded from this study. One hundred twenty-nine (male, 24; female, 105) HCV carriers with PNAL underwent liver biopsy, 69 were followed for more than 5 years (mean follow-up period, 8.5 years; Table 1), and 35 underwent serial liver biopsy during 3.4–13.4 years after the first biopsy. Formalin-fixed liver specimens were stained with hematoxylin-eosin, Masson's trichrome stain, and Perls' Prussian blue stain. Steatosis was defined as having fat droplets in more than 10% of hepatocytes. Serum ferritin and thioredoxin (oxidative stress marker)[10] levels were determined. Quantification and determination of HCV RNA and genotyping were established.

Abbreviations used in this paper: HCC, hepatocellular carcinoma; IFN, interferon; NR, nonresponder; PNAL, persistently normal serum ALT levels; SR, sustained responder; SVR, sustained viral responder; TR, transient biochemical responder.

1542-3565/05/$30.00

PII: 10.1053/S1542-3565(05)00701-9

Table 1. Characteristics of 129 HCV Carriers With PNAL Who Underwent Liver Biopsy and 69 HCV Carriers With PNAL Followed > 5 Years

	HCV carriers with PNAL (N = 129)	Those followed > 5 y (N = 69)
Follow-up period (*y*)	5.7 ± 3.6	8.5 ± 2.4
Sex (male/female)	24/105	8/61
Age (*y*)	48 (21–77)	45 (29–71)
Men	49.8 ± 16.4	42.3 ± 14.9
Women	47.2 ± 12.5	46.63 ± 11.6
ALT (*U/L*)	8–30	9–30
Men	22.5 ± 5.7	21.1 ± 5.4
Women	21.6 ± 4.8	22.3 ± 5.1
PLT (×10^4/*mL*)	15–31	15–31
Men	20.3 ± 4.4	20.9 ± 5.3
Women	21.8 ± 4.4	21.2 ± 4.0
Ferritin (*ng/mL*)	5–225	5–225
Men	76.2 ± 53.5	84.6 ± 59.2
Women	60.0 ± 43.3	66.6 ± 52.5
HCV RNA (*KIU/mL*)	6–3350	22–2100
G1 (n = 58)	648.9 ± 622.57[a]	595.1 ± 561.1[b] (N = 32)
G2 (n = 45)	356.2 ± 628.8	211.0 ± 219.2 (N = 27)
Mixed & unclassified	6–1994	

NOTE. Values were expressed as mean ± standard deviation. *P* values were calculated by Mann-Whitney *U* analysis with correction for tie.
PLT, platelet count; KIU, kilo international unit.
[a]*P* = .0012 (G1 vs G2).
[b]*P* = .0006 (G1 vs G2).

One thousand two hundred forty-six chronic hepatitis C patients (stage F1: 231, F2: 638, F3: 336, F4: 41) received 24 weeks of IFN therapy within 6 months after liver biopsy and were followed for more than 2 years (mean follow-up period, 7.7 years). All of these patients underwent liver biopsy before IFN therapy, and their stages of fibrosis were evaluated.

Another study included 3626 patients (2344 men, 1282 women) with chronic hepatitis C who had received IFN monotherapy. Patients with positive HBsAG and/or with high titer of anti-HBcAG were excluded in these 2 studies. Cox proportional hazards analysis was used to compare SRs (N = 1197; 776 men, 421 women) who developed HCC in a multicenter, retrospective cohort study. Mean follow-up was 5.9 ± 1.9 years.

Data values are expressed as medians with interquartile ranges. We compared continuous variables by using the Mann-Whitney *U* test. The Kruskal-Wallis test was used for multiple group comparisons, and Spearman correction coefficient was used to examine the relationship between groups. Frequency analysis was performed with the χ^2 test and Fisher exact test. *P* values of less than .05 were considered significant.

Results

Of the 129 patients with PNAL, 17 had normal liver histology, 102 exhibited minimal to mild chronic hepatitis, and only 10 patients exhibited F2 and/or A2 liver histology. Steatosis (9 of 129, 7%) and iron loading (6 of 50, 12%) were at significantly lower rate in these patients compared with chronic hepatitis C patients with abnormal serum ALT. Of the 104 patients who were followed for more than 2 years (mean, 6.8 years), 90 (86.5%) exhibited abnormal ALT (≥31 U/L) at least once during the follow-up period. Approximately 30% of these 102 changed to chronic hepatitis with elevated serum ALT levels within 5 years. Of the 69 patients who were followed for more than 5 years, 10 (14%) had persistently normal ALT levels (group A) during the follow-up period, 39 (57%) had transient elevation of ALT (group B), and 20 (29%) changed to chronic hepatitis (group C). The rate of progression of fibrosis in groups A, B, and C were 0.05, 0.04, and 0.08 per year, respectively. Serum ferritin and thioredoxin levels were within the normal range; however, serum thioredoxin levels were significantly higher in chronic hepatitis and cirrhotic patients.[11] No HCC was noted in any of the 129 HCV carriers with PNAL during the follow-up period.

Of the 1246 patients who received IFN therapy, 397 showed sustained biochemical response (SR; 352 were sustained virologic responders [SVRs]), 317 were transient biochemical responders (TRs; relapsers), and 532 were nonresponders (NRs). SR rate in each stage was as follows: F1: 45%, F2: 34%, F3: 22%, and F4: 10%. HCC developed in 4 of F1 patients, in 43 in F2, in 92 in F3, and in 19 in F4. The annual incidences of HCC in NRs in each stage were 0.5% in F1, 1.8% in F2, 5.2% in F3, and 8.6% in F4. The annual incidences of HCC in SRs, TRs, and NRs were 0.3%, 1.1%, and 3.3%, respectively (Table 2). There were significant differences in the development of HCC between SRs versus TRs, TRs versus NRs, and SRs versus NRs.

Among 1197 SR patients, HCC was detected in 27 patients (25 men, 2 women) (Figure 1) who were mainly male patients older than 55 years with advanced stage liver histology at entry of IFN therapy. Of the 27 HCC patients, 25 were SVRs and 2 were SRs.

Table 2. Annual Incidence of HCC After IFN Therapy (Follow-up Period, 7.7 Years)

Stage	SR (n = 397)	TR (n = 317)	NR (n = 532)
F1 (n = 231)	1/104 (0.2%)	1/72 (0.2%)	2/55 (0.5%)
F2 (n = 638)	1/216 (0.1%)	8/170 (0.6%)	34/252 (1.8%)
F3 (n = 336)	6/73 (1.1%)	16/68 (3.4%)	69/195 (5.2%)
F4 (n = 41)	0/4 (0%)	2/7 (4.9%)	17/30 (8.5%)
Total 1246	8/397 (0.3%)	23/317 (1.1%)	96/532 (3.3%)

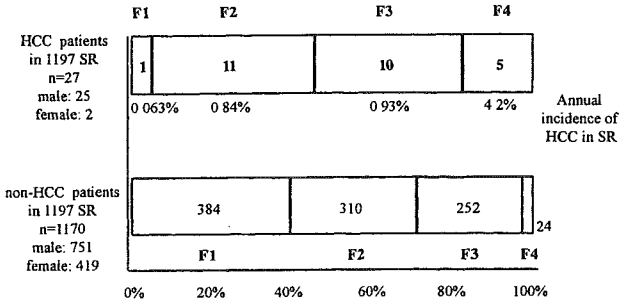

Figure 1. Fibrosis (F) stage of patients who developed HCC in 1197 SRs compared with those without HCC.

Discussion

Most HCV-infected patients with PNAL exhibited minimal to mild chronic hepatitis, had normal serum ferritin and thioredoxin levels, and exhibited slow progression of liver fibrosis when normal range of serum ALT was defined as ≤30 U/L. No HCC was detected in any of the patients. However, approximately 30% of them changed to chronic hepatitis with abnormal serum ALT within 5 years.

IFN therapy lowered the rate of progression to HCC in both SRs and TRs. The development of HCC increased in proportion to the progress of hepatic fibrosis in both TRs and SRs. The inhibition of the development of HCC was almost the same in TRs with SR for 4–5 years after IFN therapy[2]; however, after that the occurrence of HCC gradually increased in TRs.

HCC was detected in 27 patients among 1197 SRs; 25 were men and 2 were women. SRs among male patients older than 55 years with advanced stage liver fibrosis have a risk of development of HCC after IFN therapy.[12]

The present results indicate that most HCV carriers with PNAL have a good prognosis with a low risk of developing HCC. Antiviral therapy for these patients should take into consideration the follow-up results of blood chemistry and liver histology.[13]

The results of IFN therapy demonstrate that a transient biochemical response in chronic hepatitis C patients suppressed the progression of liver disease, resulting in the delay in the development of HCC; however, these effects were within 5 years. Longer therapy might be needed for the longer inhibition of the development of HCC.

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Original Paper

Intervirology

Intervirology 2005;48:372–380
DOI: 10.1159/000086064

Received: November 10, 2004
Accepted after revision: December 9, 2004

Association of Amino Acid Substitution Pattern in Core Protein of Hepatitis C Virus Genotype 1b High Viral Load and Non-Virological Response to Interferon-Ribavirin Combination Therapy

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Key Words
Hepatitis C virus · Genotype 1b · Albumin · Core region · Interferon sensitivity-determining region · Interferon · Ribavirin · Non-virological responder · Viral kinetics

Abstract
Objective: Patients with high titer (≥100 kIU/ml) of hepatitis C virus (HCV) genotype 1b do not achieve highly sustained virological response rates to combination therapy with interferon plus ribavirin. Non-virological responders (NVRs, namely ultimate resistant cases) who do not achieve HCV-RNA negativity during treatment are also encountered. We investigated the pretreatment virological features of NVRs. ***Methods:*** We evaluated 50 consecutive Japanese adults with high titer of HCV genotype 1b who received combination therapy for 48 weeks. We investigated the pretreatment substitution patterns in amino acids 1–191 of the core region and amino acids 2209–2248 of NS5A, and early viral kinetics. ***Results:*** Overall, a non-virological response was noted in 12 (24%) patients. Multivariate analysis identified serum albumin <3.9 g/dl, substitutions of amino acid 70 in the core region, and substitutions of amino acid 91 as independent and significant factors associated with a non-virological response. Especially, substitutions of arginine (R) by glutamine (Q) at amino acid 70, and/or leucine (L) by methionine (M) at amino acid 91 were significantly more common in NVRs. The falls in HCV-RNA levels during treatment in patients with specific substitutions in the core region were significantly less than in those without such substitutions. ***Conclusions:*** Our results suggest that serum albumin and amino acid substitution patterns in the core region in patients with high titers of HCV genotype 1b may have an effect on combination therapy in NVRs. Further large-scale studies are required to examine the role of amino acid substitutions specific to a non-virological response to combination therapy.

Introduction

Hepatitis C virus (HCV) usually causes chronic infection which can result in liver cirrhosis and hepatocellular carcinoma (HCC) [1–4]. The aims of interferon (IFN) therapy for chronic hepatitis C include a reduction in the risk of development of HCC and liver-related death by

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viral clearance, and then by normalization of alanine aminotransferase (ALT) even if viral clearance cannot be achieved [5].

The most effective initial therapy for viral clearance is the combination of IFN and ribavirin (RBV) administered for 48 weeks [6, 7]. In Japan, about 70% of patients with chronic hepatitis C are infected with HCV genotype 1b, and a sustained virological response (SVR) to IFN monotherapy for 24 weeks is as low as 10–20% in patients with genotype 1b infection [8–11]. Moreover, patients with a high titer of genotype 1b (≥100 kIU/ml) do not achieve high SVR rates (<50%), even when the most effective combination treatment (IFN plus RBV) is administered for 48 weeks [6, 7]. Furthermore, in genotype 1b, we also often encounter non-virological responders (NVRs) who do not achieve HCV-RNA negativity as determined by polymerase chain reaction (PCR) during treatment, compared with only 1.0% (non-virological response rate) of patients infected with genotype 2a and treated with IFN monotherapy [12]. The underlying mechanism(s) of the different virological responses to treatment in patients with 1b strain infection is still unclear. Hence, in the present study, we investigated the pretreatment virological features of NVRs.

The present study included 50 consecutive Japanese adults with chronic hepatitis C of genotype 1b and a high viral load who received combination therapy for 48 weeks. The aims of the study were: (1) to investigate the rate of non-virological responses in this group; (2) to analyze the predictive factors associated with a non-virological response, including pretreatment virological features, and (3) to examine the pretreatment virological features associated with early viral kinetics. Previous studies have shown that the HCV core region might be associated with resistance to IFN therapy involving the Jak-STAT signaling cascade [13–16], and have also shown that the number of substitutions in amino acids 2209–2248 (IFN-sensitivity determining region, ISDR, of NS5A in HCV genotype 1b) [17, 18] might be associated with the efficacy of IFN therapy and viral load. Therefore, we analyzed the amino acid substitutions of the core region and NS5A in patients with genotype 1b and high viral load to identify the virus-related factors apart from the genotype and viral load.

Materials and Methods

Study Population

Fifty-seven HCV-infected adult Japanese patients were consecutively recruited into the study protocol of combination therapy with IFN (peginterferon (PEG)-IFNα-2b or IFNα-2b) plus RBV for 48 weeks between 2001 and 2004 at Toranomon Hospital, Tokyo, Japan. Among these, 50 patients were selected in the present study based on the following criteria. (1) They were negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp, Emerville, Calif., USA), and positive for HCV-RNA qualitative analysis with PCR (Amplicor, Roche Diagnostic Systems, Pleasanton, Calif., USA). (2) They were naive to RBV therapy. (3) They were infected with HCV genotype 1b alone. (4) Each had a high viral load (≥100 kIU/ml) by quantitative analysis of HCV-RNA with PCR (Amplicor HCV-RNA kit, version 2.0, Roche Diagnostics) within the preceding 2 months of enrolment. (5) Each had chronic hepatitis, without cirrhosis or HCC, as confirmed by biopsy examination within the preceding 12 months of enrolment. (6) They had abnormal serum ALT levels (the upper limit of normal for ALT, 45 IU/l) within the preceding 2 months of enrolment. (7) In each patient, the hemoglobin (Hb) concentration was ≥12.0 g/dl, platelet count ≥ $100 \times 10^3/mm^3$, and neutrophil count ≥ $1.5 \times 10^3/mm^3$ within the preceding 2 months of enrolment. (8) Their body weight was >40 kg. (9) All were free of co-infection with human immunodeficiency virus. (10) None had been treated with antiviral or immunosuppressive agents within the preceding 3 months of enrolment. (11) None was an alcoholic; lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (12) None had diabetes, other forms of hepatitis, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (13) None of the females was pregnant or lactating. (14) All accepted treatment for 24 weeks or more as outlined in the study protocol, as well as repeated evaluation of HCV-RNA levels during treatment (at least once every month). (15) Each signed a consent form of the study protocol that had been approved by the Human Ethics Review Committee of Toranomon Hospital.

With regard to the treatment protocol, 34 (68.0%) patients received the PEG-IFNα-2b treatment protocol at dose of 1.5 μg/kg subcutaneously each week plus oral RBV at 600–800 mg/day for 48 weeks. The remaining 16 (32.0%) patients received 6 million units of IFNα-2b intramuscularly each day for 48 weeks (6 times per week for initial 2 weeks, followed by 3 times per week for 46 weeks), and oral RBV at a dose of 600–800 mg/day for 48 weeks. The RBV dose was adjusted according to body weight (600 mg for weight ≤60 kg, and 800 mg for weight >60 kg).

Table 1 summarizes the profiles and data of the 50 patients at the commencement of combination therapy with IFN plus RBV. They included 31 men and 19 women, aged 20–65 (median 53) years. The median total duration of treatment was 48 (range 28–48) weeks. In 14 of the 50 (28.0%) patients, the dose of RBV was reduced during treatment due to a fall in Hb concentration.

Patients who remained positive for HCV-RNA based on quantitative and/or qualitative analyses with PCR during and at the end of combination therapy were defined as NVRs (namely ultimate resistant cases), while the other patients who could achieve negative HCV-RNA by qualitative analysis with PCR during and/or at the end of treatment were defined as virological responders (VRs).

Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for ALT and HCV-RNA levels. The serum samples were frozen at −80°C within 4 h of collection and were thawed at the time of measurement. HCV

genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of NS5 region [19]. HCV-RNA levels were measured quantitatively by PCR (Amplicor HCV-RNA kit, version 2.0, Roche Diagnostics) at least once every month before, during, and after therapy. The lower limit of the assay was 0.5 kIU/ml. Samples collected during and after therapy that showed undetectable levels of HCV-RNA (<0.5 kIU/ml) were checked also by qualitative PCR (Amplicor, Roche Diagnostic Systems), which has a higher sensitivity than quantitative analysis, and the results are expressed as positive or negative. The lower limit of the assay was 100 copies/ml.

Histopathological Examination of Liver Biopsies

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens for examinations contained 6 or more portal areas. Histopathological diagnosis was confirmed by an experienced liver pathologist (H.K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on histological assessment according to the scoring system of Desmet et al. [20]. Hepatocyte steatosis was graded as either none (absent), mild (<1/3 of hepatocytes involved), moderate (>1/3 but <2/3 of hepatocytes involved), or severe (>2/3 of hepatocytes involved) [21].

Nucleotide Sequencing of the Core and NS5A Gene

We determined the sequences of amino acids 1–191 in the core and amino acids 2209–2248 (ISDR) in the NS5A by the direct sequencing method using pretreatment sera of 50 patients. These sequences were compared with the consensus sequence of genotype 1b, which was determined by comparing the sequences obtained in this study and prototype sequence (HCV J) [22]. HCV-RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo). Nucleic acids were amplified by PCR using the following primers. (a) Nucleotide sequences of the core region: the first-round PCR was performed with CC11 (sense, 5′-GCC ATA GTG GTC TGC GGA AC-3′) and e14 (antisense, 5′-GGA GCA GTC CTT CGT GAC ATG-3′) primers, and the second-round PCR with CC9 (sense, 5′-GCT AGC CGA GTA GTG TT-3′) and e14 (antisense) primers. (b) Nucleotide sequences of ISDR in NS5A: the first-round PCR was performed with ISDR1 (sense, 5′-ATG CCC ATG CCA GGT TCC AG-3′) and ISDR2 (antisense, 5′-AGC TCC GCC AAG GCA GAA GA-3′) primers, and the second-round PCR with ISDR3 (sense, 5′-ACC GGA TGT GGC AGT GCT CA-3′) and ISDR4 (antisense, 5′-GTA ATC CGG GCG TGC CCA TA-3′) primers (hemi-nested PCR and nested PCR). All samples were initially denatured at 95°C for 15 min. The 35 cycles of amplification were set as follows: denaturation for 1 min at 94°C, annealing of primers for 2 min at 55°C, and extension for 3 min at 72°C with an additional 7 min for extension. Then 1 μl of the first PCR product was transferred to the second PCR. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo).

To avoid false-positive results, the procedures recommended by Kwok and Higuchi [23] to prevent contamination were strictly applied to these PCR assays. No false-positive results were observed in this study.

Viral Kinetic Study

Viral kinetic study was evaluated at three time points (4, 8 and 12 weeks during treatment). Falls in HCV-RNA levels from baseline were expressed using $\log_{10}$ of viral loads at each time point, in comparison with the pretreatment viral load. For data analysis, we used the $\log_{10}$ of the cutoff value (500 IU/ml) for HCV-RNA values below the limit of detection.

Table 1. Patient profile and laboratory data at commencement of combination therapy with interferon plus ribavirin for 48 weeks in 50 patients infected with HCV genotype 1b

Demographic data	
Number	50
Sex, M/F	31/19
Age, years[a]	53 (20–65)
History of blood transfusion	14 (28.0%)
Family history of liver disease	16 (32.0%)
Body mass index, kg/m^2[a]	23.2 (18.7–32.0)
Laboratory data[a]	
Serum alanine aminotransferase, IU/l	97 (35–276)
Serum albumin, g/dl	3.8 (3.1–4.2)
Hemoglobin, g/dl	14.4 (12.0–17.4)
Platelet count, $\times 10^4$/mm^3	17.4 (10.1–30.9)
ICG R15, %[b]	13 (7–41)
Serum iron, μg/dl	140 (52–308)
Serum ferritin, μg/l	150 (<10–644)
Creatinine clearance, ml/min	101 (46–142)
Viremia level, KIU/ml	710 (49–2,800)
Number of amino acid substitutions in ISDR (0/1–3/≥4)	27/20/3
Histological findings	
Stage (F1/F2/F3)[c]	31/15/4
Hepatocyte steatosis (none/mild/moderate/severe)	3/40/7/0
Treatment	
PEG-IFNα-2b/IFNα-2b	34/16
Ribavirin dose, mg/kg[a]	11.3 (9.7–14.2)

ALT levels were abnormal (the upper limit of normal for ALT; 45 IU/l) and viremia levels were high titer (≥100 kIU/ml), when all patients were recruited in this study. Normal reference ranges: 3.9–5.2 g/dl for albumin.

[a] Expressed as median (range).

[b] ICG R15: indocyanine green retention rate at 15 min.

[c] Stage of chronic hepatitis by Desmet et al. [20].

Statistical Analysis

Non-parametric tests were used to analyze the decline in HCV-RNA levels and amino acid substitutions in HCV core and NS5A between the each groups, including the Mann-Whitney U test, χ^2 test and Fisher's exact probability test. Univariate and multivariate logistic regression analyses were used to determine the factors that significantly contributed to a non-virological response. We also calculated the odds ratios and 95% confidence intervals (95% CI). All p values of <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. Potential predictive factors associated with NVR included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, ALT, albumin, Hb, platelet count, indocyanine green retention rate at 15 min (ICG R15), serum iron, serum ferritin, creatinine clearance, viremia level, pathological staging, hepatocyte steatosis, type of IFN, RBV dose according to body weight, treatment term, dose reduction, and pretreatment amino acid substitution in the core and ISDR of NS5A. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

Virological Response Rates by Combination Therapy

The virological response could be evaluated in all 50 patients. In this study, 38 of 50 (76.0%) patients achieved a virological response while the remaining 12 (24.0%) patients were considered NVRs.

Predictive Factors Associated with a Non-Virological Response in Multivariate Analysis

We then analyzed the data of the whole population sample to determine those factors that could predict a non-virological response. Univariate analysis identified 5 parameters that tended to or significantly influenced the non-virological response. These included serum albumin ($p = 0.008$), presence of amino acid substitution in HCV core in the pretreatment sample (substitution of amino acid 70, $p = 0.003$, and amino acid 91, $p = 0.044$), RBV dose according to body weight ($p = 0.044$), and serum ferritin ($p = 0.095$).

Multivariate analysis identified three parameters that independently influenced the non-virological response, including serum albumin ($p = 0.004$), substitutions of amino acids 70 ($p = 0.013$) and 91 ($p = 0.016$; table 2).

Treatment Efficacy according to Substitution Patterns in Amino Acids of HCV Core

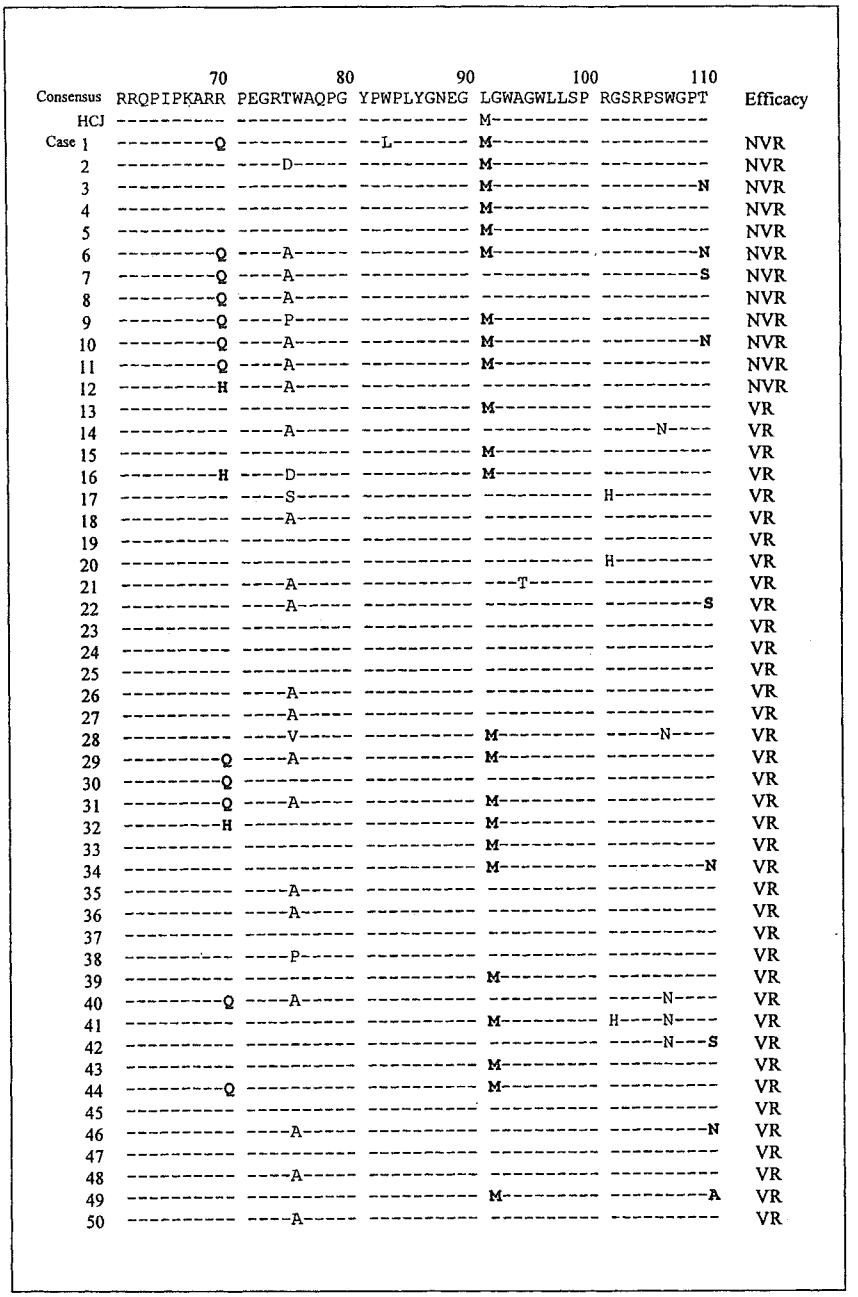
Figure 1 shows the sequences of amino acids 61–110 of the HCV core in 50 patients at the commencement of combination therapy. Substitutions at amino acid 70 of the HCV core were significantly more frequent in NVRs (n = 8, 66.7%) than VRs (n = 7, 18.4%; $p = 0.003$). Similarly, substitutions at amino acid 91 were significantly more frequent in NVRs (n = 9, 75.0%) than VRs (n = 14, 36.8%; $p = 0.044$). Furthermore, dual substitutions at amino acids 70 and 91 were significantly more frequent in NVRs (n = 5, 41.7%) than VRs (n = 5, 13.2%; $p = 0.046$). Thus, substitutions at amino acid(s) 70 and/or 91 were found in all 12 (100%) NVRs while only 16 (42.1%) of the VRs had such substitutions ($p < 0.001$). There were no significant differences in other substitution sites and treatment efficacy between NVR and VR groups (table 3).

At amino acid 70, the substitution in which arginine (R) was replaced by glutamine (Q) was significantly more frequent in NVRs (n = 7, 58.3%) than VRs (n = 5, 13.2%; $p = 0.004$). At amino acid 91, the substitution in which leucine (L) was replaced by methionine (M) was significantly more frequent in NVRs (n = 9, 75.0%) than VRs (n = 14, 36.8%; $p = 0.044$). At amino acid 110, the substitution in which threonine (T) was replaced by asparagine (N) was significantly more frequent in NVRs (n = 3, 25.0%) than VRs (n = 2, 5.3%; $p = 0.082$). Substitutions Q–M instead of R–L at amino acids 70 and 91 were significantly more frequent in NVRs (n = 5, 41.7%) than VRs (n = 3, 7.8%; $p = 0.014$). Thus, 11 (91.7%) NVRs and 16 (42.1%) VRs ($p = 0.003$) had a substitution of Q at amino acid 70 and/or M at amino acid 91. There were no significant differences in other substitution patterns and treatment efficacy between NVRs and VRs (table 3).

Table 2. Factors associated with non-virological response to combination therapy with interferon plus ribavirin for 48 weeks in 50 patients infected with HCV genotype 1b, identified by multivariate analysis

Factor	Category	Odds ratio (95% confidence interval)	p
Albumin, g/dl	1: <3.9	1	
	2: ≥3.9	0.009 (0.000–0.227)	0.004
Substitution of aa 70	1: Absent	1	
	2: Present	22.2 (1.905–258.3)	0.013
Substitution of aa 91	1: Absent	1	
	2: Present	19.5 (1.737–219.3)	0.016

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown. aa = Amino acid.



	70	80	90	100	110	
Consensus	RRQPIPKARR	PEGRTWAQPG	YPWPLYGNEG	LGWAGWLLSP	RGSRPSWGPT	Efficacy
HCJ	----------	----------	----------	M---------	----------	
Case 1	---------Q	----------	--L-------	M---------	----------	NVR
2	----------	----D-----	----------	M---------	----------	NVR
3	----------	----------	----------	M---------	---------N	NVR
4	----------	----------	----------	M---------	----------	NVR
5	----------	----------	----------	M---------	----------	NVR
6	---------Q	----A-----	----------	M---------	---------N	NVR
7	---------Q	----A-----	----------	----------	---------S	NVR
8	---------Q	----A-----	----------	----------	----------	NVR
9	---------Q	----P-----	----------	M---------	----------	NVR
10	---------Q	----A-----	----------	M---------	---------N	NVR
11	---------Q	----A-----	----------	M---------	----------	NVR
12	---------H	----A-----	----------	----------	----------	NVR
13	----------	----------	----------	M---------	----------	VR
14	----------	----A-----	----------	----------	-----N----	VR
15	----------	----------	----------	M---------	----------	VR
16	---------H	----D-----	----------	M---------	----------	VR
17	----------	----S-----	----------	----------	H---------	VR
18	----------	----A-----	----------	----------	----------	VR
19	----------	----------	----------	----------	----------	VR
20	----------	----------	----------	----------	H---------	VR
21	----------	----A-----	----------	---T------	----------	VR
22	----------	----A-----	----------	----------	---------S	VR
23	----------	----------	----------	----------	----------	VR
24	----------	----------	----------	----------	----------	VR
25	----------	----------	----------	----------	----------	VR
26	----------	----A-----	----------	----------	----------	VR
27	----------	----A-----	----------	----------	----------	VR
28	----------	----V-----	----------	M---------	-----N----	VR
29	---------Q	----A-----	----------	M---------	----------	VR
30	---------Q	----------	----------	----------	----------	VR
31	---------Q	----A-----	----------	M---------	----------	VR
32	---------H	----------	----------	M---------	----------	VR
33	----------	----------	----------	M---------	----------	VR
34	----------	----------	----------	M---------	---------N	VR
35	----------	----A-----	----------	----------	----------	VR
36	----------	----A-----	----------	----------	----------	VR
37	----------	----------	----------	----------	----------	VR
38	----------	----P-----	----------	----------	----------	VR
39	----------	----------	----------	M---------	----------	VR
40	---------Q	----A-----	----------	----------	-----N----	VR
41	----------	----------	----------	M---------	H----N----	VR
42	----------	----------	----------	----------	-----N---S	VR
43	----------	----------	----------	M---------	----------	VR
44	---------Q	----------	----------	M---------	----------	VR
45	----------	----------	----------	----------	----------	VR
46	----------	----A-----	----------	----------	---------N	VR
47	----------	----------	----------	----------	----------	VR
48	----------	----A-----	----------	----------	----------	VR
49	----------	----------	----------	M---------	---------A	VR
50	----------	----A-----	----------	----------	----------	VR

Fig. 1. Sequences of amino acids 61–110 in the core region at the commencement of combination therapy in 50 patients infected with high HCV viral load genotype 1b. Dashes indicate amino acids identical to the consensus sequence of genotype 1b, and substituted amino acids are shown by standard single-letter codes. The amino acid patterns at positions that are probably associated with sensitivity to therapy are shown in boldface characters. NVR = Non-virological responder; VR = virological responder.

Viral Kinetics according to Substitution Patterns in Amino Acids of HCV Core

Table 4 shows HCV-RNA levels at 4, 8, and 12 weeks relative to baseline as a function of pretreatment amino acid substitutions in the core region. The fall in HCV-RNA level at each time point was significantly lower in patients with specific substitution patterns (Q at amino acid 70, M at amino acid 91, N at amino acid 110, Q–M at amino acid 70 and 91, Q at amino acid 70 and/or M at amino acid 91) than in those without them.

Table 3. Amino acid substitutions in the core region in non-virological responders (NVR) and virological responders (VR) to combination therapy of interferon plus ribavirin for 48 weeks in 50 patients infected with HCV genotype 1b

	NVR (n = 12)	VR (n = 38)	p*
Presence of substitution site			
aa 70	8 (66.7%)	7 (18.4%)	0.003
aa 91	9 (75.0%)	14 (36.8%)	0.044
aa 70 and 91	5 (41.7%)	5 (13.2%)	0.046
aa 70 and/or 91	12 (100%)	16 (42.1%)	<0.001
Presence of substitution pattern			
Q at aa 70	7 (58.3%)	5 (13.2%)	0.004
M at aa 91	9 (75.0%)	14 (36.8%)	0.044
N at aa 110	3 (25.0%)	2 (5.3%)	0.082
Q-M at aa 70 and 91	5 (41.7%)	3 (7.8%)	0.014
Q at aa 70 and/or M at aa 91	11 (91.7%)	16 (42.1%)	0.003

Q = Glutamine; M = methionine; N = asparagine; aa = amino acid.
* NVR vs. VR (Fisher's exact probability test).

Table 4. Decline levels of HCV-RNA from baseline at 4, 8 and 12 weeks according to the amino acid substitutions in the core region during combination therapy of interferon plus ribavirin for 48 weeks in 50 patients infected with HCV genotype 1b

Presence of substitution pattern	Decline levels of HCV-RNA from baseline, log_{10} IU/ml[1]		
	4 weeks	8 weeks	12 weeks
Q at aa 70			
Absent[2]	2.49 (–0.024 to 3.41) [a]	3.02 (0.25 to 3.41) [b]	2.98 (0.30 to 3.45) [c]
Present	0.58 (0.11 to 3.13)	1.18 (–0.095 to 3.16)	1.99 (0.34 to 3.19)
M at aa 91			
Absent	2.49 (0.12 to 3.41) [d]	3.14 (1.69 to 3.41) [e]	3.13 (0.49 to 3.45) [f]
Present	0.85 (–0.024 to 3.16)	1.56 (–0.095 to 3.41)	2.40 (0.30 to 3.41)
N at aa 110			
Absent	2.36 (0.10 to 3.41) [g]	3.02 (–0.095 to 3.41) [h]	2.96 (0.48 to 3.45) [i]
Present	0.28 (–0.024 to 0.86)	0.32 (0.25 to 1.43)	0.70 (0.30 to 2.46)
Q-M at aa 70 and 91			
Absent	2.49 (–0.024 to 3.41) [j]	3.04 (0.25 to 3.41) [k]	2.98 (0.30 to 3.45) [l]
Present	0.58 (0.11 to 2.34)	0.50 (–0.095 to 2.34)	1.99 (0.34 to 3.19)
Q at aa 70 and/or M at aa 91			
Absent	2.49 (0.88 to 3.41) [m]	3.11 (1.69 to 3.41) [n]	3.18 (2.40 to 3.45) [o]
Present	0.85 (–0.024 to 3.16)	2.01 (–0.095 to 3.41)	2.40 (0.30 to 3.41)

Q = Glutamine; M = methionine; N = asparagine; aa = amino acid.
[1] Decline levels of HCV-RNA from baseline are shown in log_{10} of viral loads at each time point in comparison to pretreatment viral loads. For HCV-RNA quantitative values below the limit of detection, we used the log_{10} of the cutoff value (500 IU/ml) for data analysis. Data are expressed as median (range).
[2] Absent vs. Present of substitution pattern (Mann-Whitney U test): [a] p = 0.025; [b] p = 0.019; [c] p = 0.011; [d] p = 0.049; [e] p = 0.001; [f] p = 0.007; [g] p = 0.010; [h] p = 0.002; [i] p = 0.004; [j] p = 0.018; [k] p = 0.001; [l] p = 0.019; [m] p = 0.028; [n] p = 0.006; [o] p = 0.001.

Discussion

The main finding of the present study was that resistance to PEG-IFNα-2b+RBV or IFNα-2b+RBV combination therapy in patients with chronic hepatitis C genotype 1b and high viral load was partly influenced by serum albumin, substitutions of amino acids 70 and 91.

We previously showed that serum albumin was a negative predictor of SVR to IFN monotherapy in HCV patients, based on multivariate analysis [12]. Serum proteins including albumin are synthesized by hepatocytes, and falls in their concentrations usually reflect decreased hepatic synthesis although changes in plasma volume could also contribute to such falls. Advanced liver fibrosis is usually associated with decreased hepatic synthesis and low levels of serum albumin [24]. On the other hand, the absence of advanced liver fibrosis is a predictor of SVR to IFN monotherapy and combination therapy of IFN/RBV [11, 25–27]. This report on VRs showed that a milder form of liver fibrosis was not a positive predictor of response to combination therapy, compared with high levels of serum albumin [24]. These discrepant findings may be due to one or more factors. The first reason is probably related to the method used for evaluation; the degree of liver fibrosis roughly reflects liver function but can only be assessed using a three-stage (F1, F2, F3) system, in contrast to the serum albumin level. Thus, serum albumin might reflect liver function more sensitively than the degree of liver fibrosis. Furthermore, this finding showed that the ability of the liver to synthesize serum proteins including albumin might contribute to the observed response to treatment more than the degree of liver fibrosis. The second reason is probably related to the design of our study based on comparison between a virological and a non-virological response, rather than a SVR and a non-SVR. Our study based on multivariate analysis is the first to identify serum albumin as a predictor of a non-virological response in patients on 48-week IFN/RBV combination therapy.

IFN-α and IFN-β bind to the type-I IFN receptor, and one major pathway in type-I IFN signaling involves the Jak-STAT signaling cascade [13, 28–37]. Previous studies reported that the HCV core region might be associated with resistance to the antiviral actions of IFN therapy. Blindenbacher et al. [14] showed that STAT signaling was strongly inhibited in liver cells of HCV core transgenic mice. Bode et al. [15] showed that HCV core protein induced the expression of the suppressor of cytokine signaling-3 and inhibited activation, tyrosine phosphorylation, and nuclear translocation of STAT1, which might impair the antiviral actions of IFNs in HepG2 cells. Furthermore, Mélen et al. [16] indicated that IFN-induced nuclear accumulation of STAT1 was almost completely blocked and STAT2 was partially blocked in cell lines expressing high levels of HCV core protein. Our study identified amino acid substitutions in HCV core as a predictor of a non-virological response to 48-week IFN/RBV combination therapy based on multivariate analysis. This result suggests that substitutions of amino acids in the HCV core region might be associated with resistance to the antiviral actions of IFN therapy involving the Jak-STAT signaling cascade.

Since combination therapy could induce hemolytic anemia and possibly other major side effects [6], it is important to identify resistant patients, especially NVRs among non-sustained virological responders, early during therapy with the intent of revising the treatment regimen. In fact, we were able to revise or terminate treatment before completion of the full course of combination therapy for 48 weeks and spare patients from receiving unnecessary treatment based on consideration of risks/benefits. Our study indicated that falls in HCV-RNA levels from baseline were significantly lower in patients with specific pretreatment amino acid substitution patterns in the HCV core. Thus, our study identified pretreatment virological features associated with early viral kinetics during combination therapy with IFN/RBV. Further studies are required to explore the relationship between virological features and differences in viral kinetics.

In conclusion, our results suggest that albumin levels and amino acid substitution patterns in the core region in patients with a high titer of HCV genotype 1b might determine a non-virological response to combination therapy. One limitation of this study was that we did not examine other viral factors, such as amino acid substitutions in areas other than the core region and ISDR of HCV genome, as well as other host factors such as IFN-inducible protein kinase, MxA and 2′,5′-OAS protein [28–31, 37–42], although they should be investigated together with other factors in future studies. Moreover, further large-scale prospective studies are necessary to investigate whether our results also explain resistance to IFN-RBV combination therapy.

Acknowledgment

This study was supported in part by a grant-in-aid from the Ministry of Health, Labor and Welfare, Japan.

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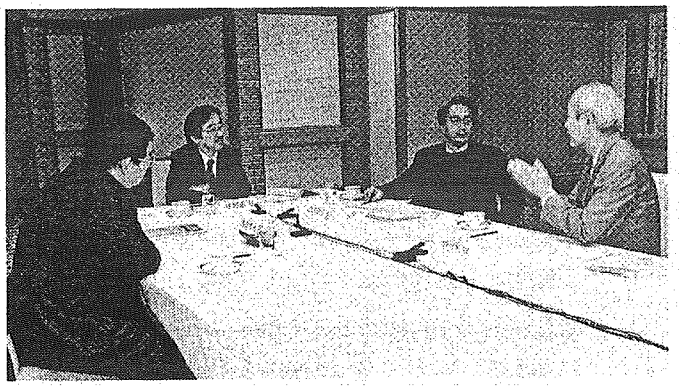
座談会

肝炎診療を見直す



井廻　道夫　昭和大・第２内科＝司会
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三代　俊治　東芝病院
（発言順）

平成17年3月1日（火）収録

井廻（司会）　この数年でウイルス肝炎に対する治療法がだいぶ変わり，新しい薬が入ってきました．そしてＣ型肝炎，Ｂ型肝炎の診断指針，治療指針が新しくできています．

また，最近は生活習慣病として非アルコール性脂肪肝炎が話題となっていますし，もともと外来の急性肝炎と思われていたＥ型肝炎が日本にも古くから存在し，しかも劇症肝炎を起こすということが新聞などで取り上げられ，注目されています．

そこで，本日は肝炎診療を見直すということで，この分野の専門の先生方に現況をお話しいただきたいと思います．

■Ｃ型慢性肝炎の治療

●治療指針の変遷

井廻　最初にウイルス肝炎についてですが，現在は治療法が非常に進歩してきています．まずＣ型肝炎の治療について，熊田先生からお願いします．

熊田　Ｃ型肝炎ウイルスは1989年に見つかり，日本では1992年，世界に先駆けてインターフェロン療法の保険診療が行われるようになりました．当時はＣ型肝炎が治ってしまうということで注目を浴び，インターフェロン療法が盛んに行われて，大体30%の人が治癒しました．

その時点で，インターフェロンによってどういう人が治って，どういう人が治らないかという疑問が出てきて，はっきりしてきたのが，Ｃ型肝炎ウイルスはいくつかのサブタイプに分けられるということです．また，ウイルス量も測定可能になりまして，ウイルス量が100Kcopies/ml以上を高ウイルス量のＣ型肝炎，以下を低ウイルス量と分類するようになりました．

この分類で治療結果をみてみますと，グループ１（genotype 1a，1b）でウイルス量が100Kcopies/ml以上の人は非常に治りにくい．ですから当初，インターフェロン療法がよく行われて，治りやすかったのは，グループ１でウイルス量が多い人以外ということが分かりました．

そしてほぼ10年間は同じような経過でしたが，2001年12月に抗ウイルス薬リバビリ

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ンとインターフェロンとの併用療法が保険適用となり，グループ1でウイルス量が多い人にも効くということが分かって，一時，盛んに行われました．それでも治癒率は大体20%台でした．

井廻　そのときの治療期間は24週ですね．

熊田　24週です．それから，インターフェロンを週3回投与するのは大変ですが，2003年12月に週1回投与すればよいという持続型インターフェロン，ペグインターフェロンα-2aが登場しました．その1年間での効果は16%でしたが，患者さんのQOLは非常に良かったわけです．

世界ではすでに2001年，2002年ごろから，ペグインターフェロンとリバビリンの併用療法の1年間投与が最も良いということが分かっていたのですが，日本でもやっと2004年12月8日に，併用療法が可能となるペグインターフェロンα-2bが発売となり，世界の標準に追い付いたというのが現状だと思います．

井廻　先ほど，C型肝炎のサブタイプについてのお話がありましたが，日本人の内訳はどうなっていますか．

熊田　日本ではグループ1が全体の約70%です．グループ2（genotype 2a，2b）が約30%ですが，さらにグループ2のなかでも2a，2bという2つのタイプがあって，2aが約20%，2bが約10%です．日本人では，いちばん治りにくいグループ1が多いということが分かります．

井廻　グループ1で，しかも高ウイルス量の人は全体の大体どのくらいになりますか．

熊田　全体でいえば，50%ぐらいです．

●治療薬の副作用と注意点

井廻　次に，副作用についてはいかがでしょうか．

熊田　インターフェロンそのものは，副作用があることはすでによく知られていますが，特にインターフェロンαのいちばんの副作用は，うつ状態になるということがあげられます．それから女性にとっては脱毛があります．初期の副作用としては発熱や関節痛がありますが，それはそう長くは続きません．

リバビリンの副作用として最大のものは貧血です．女性でヘモグロビンが低い人は，治療を始めても途中で貧血のために中止になってしまうことがあり，それが大きな問題です．そのほか，リバビリンの副作用としては発疹，あとは脳出血が少し多いのではないかと感じています．

井廻　そういう副作用に注意しながら治療を行っていくわけですが，年齢などは関係ありますか．

熊田　日本では戦後の輸血や医療行為でC型肝炎感染が拡大したと推定されていますから，いちばん多い年代は60歳代です．そうしますと，インターフェロンの副作用にリバビリンの副作用がプラスされますから，今回の開発試験でも65歳以上のインターフェロンまたはペグインターフェロンとリバビリンの

併用療法での中止率は，インターフェロン群で約43%，ペグインターフェロン群で約29%と非常に多い．ですから，高齢者に関しては投与量を少し工夫する必要があると思います．

たとえばペグインターフェロンとリバビリン併用療法の場合，用量は体重別になっていますが，ペグインターフェロンα-2bの場合は20μgと少な目にスタートする，あるいはリバビリンに関しては200mgくらいから少な目にスタートするというように，調整しながら行わないと中止が増えてしまうと思います．

井廻　以前，インターフェロンとリバビリン併用療法のときに，脳出血の副作用がありました．新しいペグインターフェロンとリバビリンの場合もやはり同じことがいえるのでしょうか．

熊田　実際に512例の治験のなかでは幸い1例も脳出血は起こりませんでした．しかし，インターフェロンとリバビリンのときには，いわゆる脳出血の多発地域である久山町の脳出血の頻度と実際には差はなかったといわれているのですが，これも全数調査と医師からの報告調査では違いますので，実際にはリバビリンを使った場合は脳出血が多くなると思います．

ただ，市販後の副作用調査からいえることは，高血圧と糖尿病の人が圧倒的に多いということです．ですから今後は，たとえば65歳以上で，なおかつ高血圧，糖尿病がある人は，この併用療法は避けるべきではないかと思います．あとは血圧をコントロールする，血糖値をコントロールするというような工夫をしながら，注意深く慎重投与ということが必要だと思います．

井廻　そうしますと，ペグインターフェロンとリバビリンの併用は，成績は非常に良いけれども，インターフェロンとリバビリンのときと同じように，糖尿病や高血圧の人は非常に注意しながら治療していかなくてはいけないということになりますね．

熊田博光（くまだ・ひろみつ）：虎の門病院副院長，消化器科部長．昭和47年岐阜大学医学部卒業．
主研究領域／慢性肝疾患の診断と治療．

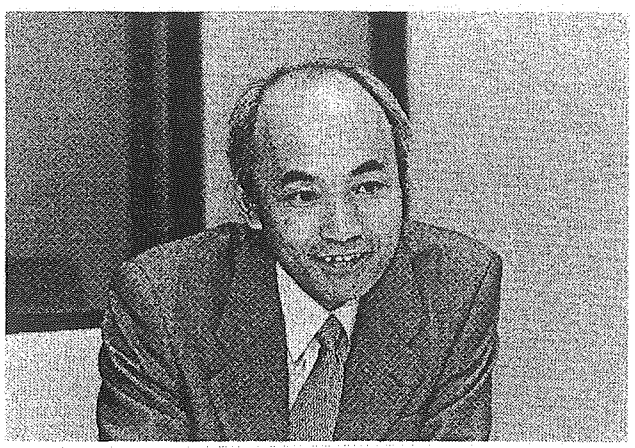
●ペグインターフェロン登場による新しい治療指針

井廻　ペグインターフェロンとリバビリンの併用療法の適応となる患者さんはどういう人ですか．

熊田　現在は，グループ1でウイルス量が100Kcopies/ml以上の人に限って認可されています．実際に治験の治療効果は約48%ですから，2人に1人が治癒してしまうということで，第一選択薬になることは間違いない．しかし，グループ2の高ウイルス量，それから低ウイルス量に関しては，まだ認可されてはいません．

ただ，グループ2の高ウイルス量に関しては，現在の公式のレポートでは，ペグインターフェロンα-2aの1年間投与が約76%ということで最も治療効果が高い．しかし，インターフェロンα-2bとリバビリンの24週で，その成績よりもさらに良い結果が出ています．

井廻　そうしますと，治療指針としては，



西原利治（さいばら・としじ）：高知大学医学部助教授（消化器病態学）．昭和55年京都大学医学部卒業．
主研究領域／自己免疫性肝疾患，NASH．

グループ2の高ウイルス量に対してはインターフェロンα-2bの24週投与ですか．

熊田　インターフェロンα-2bとリバビリンの24週を選択するか，あるいはペグインターフェロンα-2aの1年を選択するかです．副作用的には明らかにペグインターフェロンα-2aの1年投与のほうが少ないのですが，期間が倍かかるということです．

低ウイルス量に関しては，リバビリンの併用をしなくてもインターフェロン単剤で60～70%の治療効果がありますので，初回に関しては通常のインターフェロン，特に女性や老人に関してはインターフェロンβの8週間投与で十分ではないかと思います．

井廻　西原先生は，新しいペグインターフェロンとリバビリンの併用療法ができるようになって，何か今までと変わったということはありますか．

西原　明らかに発熱とか倦怠感という訴えが少なくなりまして，患者さんのコンプライアンスが良くなっていると思います．ただ，従来のインターフェロン単独の場合にはなかった初期症状が併用療法の場合は出ますので，その辺りに気を付けながら投与しています．

それから先ほど先生がおっしゃったように，貧血の問題に留意しながら治療しているのが現状だと思います．

井廻　消化器症状はリバビリンによるものが強いですね．私の患者さんでも，リバビリンで下痢が強くなって，減量せざるをえなかったことがあります．そのほか発熱の問題でいえば，ペグインターフェロンを使うことは患者さんにとってはコンプライアンスは確かに良いですよね．

ただ，実際には先ほどお話にあった副作用で併用療法ができない人もいますし，治癒するといっても48%ということは，残りの52%はどうするのかという問題がありますが，それについては何かご意見はありますか．

熊田　日本の現状をみてみますと高齢者が多いですから，一度はペグインターフェロンとリバビリンの併用療法を行ってもよいのですが，あまり副作用のことで無理押しはしないほうがよいだろうと思います．ただ，慢性肝炎は将来的に肝臓癌になることが怖いわけですから，それを避けるための治療がもう1つの選択肢としてある．

過去に，インターフェロンの量についての試験がありました．これは特にインターフェロンαで行われたのですが，300万単位，600万単位，900万単位のコントロールスタディが行われて，トランスアミナーゼの改善で最も良かったのは，少ない量だったという事実があります．

ですから，治すことができないことが分かった患者さんには少ない量，インターフェロンαを300万単位，インターフェロンβでも300万単位になると思うのですが，そのくらいの量を長期に使って，できるだけトランスアミナーゼを下げていく．それによって，

最終的には発癌予防をしていくという考えで，治療もいろいろな側面から考えないといけないと思います．

それから，インターフェロンαの場合は筋肉注射あるいは皮下注射なので，自己注射できるように今，中央社会保険医療協議会で議論されています[*1]．インスリンは自己注射が可能ですし，インターフェロンも肝炎以外はすでに自己注射になっています．肝炎の場合も自己注射ができるようになると，夜，特に10～12時くらいにインターフェロンを打つことができます．夜間は，副作用が弱いということが分かっていますので，患者さんにとってはかなりメリットが大きいのではないかと思います．

井廻　夜間は生理学的にも良いのですか．

熊田　コルチゾールの動きが良いデータになるのですが，コルチゾールは夜中の1時がいちばん低くて，朝8時が最も高いのです．インターフェロンを投与するとコルチゾールが数時間で上がってしまい，そのときに副作用が最も強く出るのです．

ですから午前中にインターフェロンを投与すると，コルチゾールが下がっていかなければならないときにコルチゾールが上がってしまう．その結果，いわゆるストレスが加わって，いろいろな副作用が出てくる．

ところが夜間に投与しますと，インターフェロンによってコルチゾールが上がってきますが，ちょうど生理現象としても上がってきますので，インターフェロンによるものと，生理的なコルチゾールの動きが一致するということで副作用が少ないといわれています．

井廻　ところで，インターフェロン単独療法でウイルスが消えなかった人は，もう効かないと考えてよいでしょうか．それともペグインターフェロンとリバビリンの併用療法を積極的に行うべきでしょうか．

熊田　国内治験のデータをみてみますと，1回のインターフェロン単独療法でウイルスが一旦消えた人に，ペグインターフェロンとリバビリンの1年間投与を行いますと，62.3%の人が治癒しています．ですから，まず第一選択としてインターフェロンを行って，ウイルスが消えたのだけれどももう少しのところで治らなかったという人は，ぜひペグインターフェロンとリバビリンの併用療法を試してみるべきだと思います．

それから初回，インターフェロンを使ったのだけれども，ウイルスが全く消えなかった人の治療効果は19.2%です．副作用のために途中で中止になった症例なども含まれていますから，やはり現在最も良いといわれている治療ですので，副作用に注意しながらチャレンジしたほうがよいと思います．

井廻　分かりました．ここまではC型肝炎について熊田先生に伺ってきましたが，西原先生は何かご発言ありますか．

三代俊治（みしろ・しゅんじ）：東芝病院研究部部長．
昭和48年東京大学医学部卒業．
主研究領域／肝炎ウイルス．

*1 平成17年5月，インターフェロンαの自己注射が認可された．

西原　肝硬変に非常に近いような症例は，血小板も少な目です．そういう患者さんの場合について，何かアドバイスをいただきたいと思うのですが．

熊田　欧米では，慢性肝炎も肝硬変も代償性肝硬変までは慢性肝炎として扱っているのですが，現実に肝硬変の人に使いますと脱落例が多いことは必須です．こういう場合はやはり量を高齢者と同じように少な目に使う．

ただ，肝硬変になりますと治療効果がだいぶ下がって，30%くらいになりますので，見極めはある程度のところでしなければいけません．一応，6か月（24週）のところで見極めをしますが，ウイルスの初期の下がり方が悪ければ，これは無理である．ですから肝硬変であれば，その見極めは早くしたほうがよいと思います．

B型慢性肝炎の治療方針

井廻　では続いて，B型慢性肝炎の治療方針について伺いたいと思います．

B型肝炎も以前はインターフェロン療法，それからステロイド離脱療法などが行われていましたが，その後，抗ウイルス薬のラミブジンが登場して，治療法も大きく変わりました．しかし，ラミブジンの場合にはbreak-through hepatitisを起こします．その場合は，最近はアデフォビルという薬をラミブジンに併用して使うことができるようになりました．

ただ，B型肝炎は自然経過でもウイルスが消えることがありますし，治療はなかなか難しいと思うのですが，熊田先生はどういう方針で治療されていますか．

熊田　かつて日本では，B型肝炎にはインターフェロンの4週間投与という投与法しかありませんでした．ところが，結果的にはあまり効果がなかったというのが一般的です．考えてみれば，ウイルス量の観点からみてもB型は一般的にC型の約1,000倍とウイルス量が多いわけですから，C型でもインターフェロン6か月投与でスタートしたのに，B型が1か月投与ではとても無理な話です．

しかし，B型肝炎に関しても24週が認められ，これによってB型肝炎全体の20～30%くらいの人を，活動型のHBe抗原陽性のB型肝炎から，非活動型というか，活動が弱くなったHBe抗体陽性のB型肝炎にさせることができるということで，6か月の投与は意味があるというのが全般的な成績だと思います．

ただB型肝炎の場合は，何もしなくても治る人がいるのがC型肝炎との大きな違いで，若い世代ではすぐに治療をするかどうかが大きな問題になっています．現在，まだ日本の肝炎専門家の間でコンセンサスが得られていない状況ですが，若い人にはなるべく治療を長く続けないで，できればインターフェロンあるいはステロイド離脱療法などを使って早めに抗原を消してあげることが重要だと思います．

その年齢も，25歳あるいは35歳までは無治療が良いというように意見がさまざまですが，35歳までは自然経過でのHBe抗原の陰性化を目指すという考えの先生が多いと思います．しかし35歳を超えますと，HBe抗原が消えることはあるのですが，消えたときには肝硬変まで進行していることが多く，35歳を超えたら早めに治療をしたほうがよいと思います．

井廻　ラミブジンを投与すると，かなりの患者さんでトランスアミナーゼが下がりますし，ウイルスが測定感度以下になりますね．ただ，やめてしまうと多くの方でまたウイル

スが出てくるということがあって，使い始めると長期間使わざるをえない．そして長期間使っていると変異株が出現してきます．

幸い，アデフォビルが登場して，これはラミブジンの変異株にも効き，併用することができるということですが，35歳を過ぎてトランスアミナーゼが変動している人には積極的に使ったほうがよいですか．

熊田　そうですね．ただ，アデフォビルは，ラミブジンの変異株によるbreak-through hepatitisが起こる症例に限って認可されているのが現状です．ですから，アデフォビルの登場によって何が変わったかというと，安心してラミブジンを使えるようになったということがいちばん大きいと思います．

かつてラミブジンが使われるときに，いつまで使うかという問題が常に議論されまして，なるべく短い期間にしたほうがよいということになったのですが，残念ながらラミブジンを使っても，やめるとまた悪くなる人が全体の7～8割ということが分かっています．ですから短期でHBe抗原を消して，HBV DNAを2.6 log copies/m*l*以下にすることが目標なのですが，現実にはラミブジンを長期投与せざるをえないのです．

そして，break-through hepatitisが起こった場合は，なるべくすみやかにアデフォビルを併用することによって，HIVと同じような感じで長期に使っていくことが必要になる．

しかし，アデフォビルにもやはり副作用があって，HIVのときにアデフォビル30mg投与しますと，腎障害が出てくることが分かっています．日本ではアデフォビルは10mgになっていますが，10mgでも安易にだらだら使っていますと，クレアチニンが上がって腎障害が出てくる可能性が高いと思います．

血液データあるいは尿中のβ2-ミクログロブリンを測って，アデフォビルの量を2日に1回にするなど，調整しながら使っていかなければいけません．

井廻　幸い，ラミブジンもアデフォビルも光学異性体ですから，そういう意味ではあまり副作用を考えなくてもよいですね．

熊田　そうですね．C型肝炎のリバビリンのように，催奇形性の報告などもありません．

井廻　35歳を過ぎて肝機能が落ち着かなければラミブジンを選択ということになりますが，35歳以下で使う場合はどのようなことに気を付ければよいでしょうか．

熊田　35歳以下の場合は，ラミブジンを短期で使います．インターフェロンとラミブジンをトータル1年間投与で，半数以上がHBe抗原が消えて安定化したという欧米の報告があります．

しかしB型肝炎のもう1つ大きな問題は，C型肝炎と同じようにウイルスがgenotype（遺伝子型）によって違うことです．日本ではgenotype Cがメインで，サブメインがBですが，欧米ではgenotype AとDが多くみられます．

genotype AとDに関しては，インターフェロンやラミブジンなどあらゆる薬が効きやすいのです．日本ではなかなか効きにくいCがメインですので，35歳以下でトランスアミナーゼの変動が激しい場合は，インターフェロンとラミブジン，あるいはステロイドとラミブジンなどの併用療法を行って，できるだけ早く短期間でHBe抗原を消すことを考えたほうがよいと思います．

■食道静脈瘤を有する無症候性原発性胆汁性肝硬変の取り扱い

井廻　次に，原発性胆汁性肝硬変（primary

biliary cirrhosis ; PBC）に移りたいと思います．

突然の食道静脈瘤の出血で，実はPBCだったということが時々あります．しかも組織をみると，線維化はあるけれども肝硬変ではない．今まではPBCの無症候性と症候性を皮膚の掻痒とビリルビン値で分けて，症候性はいわゆる難病の医療費等助成の対象ですが，無症候性は対象外になっています．

ところが静脈瘤の破裂は症候性でなくても起きることがあって，最近では食道静脈瘤を有する無症候性PBCが注目されています*2．西原先生，これに関していかがでしょうか．

西原　無症候性PBCにおける食道静脈瘤あるいは胃静脈瘤の危険性を信州大学の清澤研道先生のグループが『日本消化器病学会雑誌』にご報告なさったことが，印象に残っております．「肝硬変を伴わないPBCでも，門脈圧の亢進が生じ，静脈瘤を来すことがある」とのメッセージは新鮮で，私どもの症例でも静脈瘤が十分に検索されているか検討し，38例のうち2例にF1の食道静脈瘤が認められたことを覚えております．

実際，肝硬変に至っておらず静脈瘤も認めない無症候性PBCのヘリカルCT検査では，その目でみると5%弱の症例で門脈からの側副血行路の発達が観察されます．

このような変化は，食道からの出血に直結するものではないとしても，運悪く食道や胃の静脈瘤になると，何らかの拍子に出血を来すことも十分に考えられるということです．いわゆる無症候性PBCは予後が良いので，それほど気にしなくてよいということではなくて，逆に，無症候性PBCでは門脈圧亢進症を見逃さないように診療しようという流れが定着してほしいと思います．

井廻　熊田先生の所ではどうですか．非常にたくさんの症例をもっていらっしゃると思うのですが，掻痒や黄疸などはなくて，静脈瘤だけが問題というPBC例はありますか．

熊田　多くはないのですが，実際にありますね．どうしても肝炎ウイルスに目が行ってしまって，ウイルスがいないということで安心していると，実は無症候性PBCで，突然吐血ということがあります．

基本的にはトランスアミナーゼ，γ-GTP，ALPの比が肝炎とは何か違う，脂肪肝とも違うということにまず気付いて，PBCが隠れているのではないかという意識を常に頭に入れておかなければいけません．やはり，PBCの診断を見落とさないことがいちばん大切です．

井廻　日本ではそれほど多くはありませんが，1万2,000人あるいは推定によれば2万人くらいの患者さんがいらっしゃるようです．

私も非常に苦い思い出があって，PBCということまでは診断したのですが，早期の段階だろうということで，静脈瘤はチェックしていなかったら，出血を起こしてしまったことがありました．PBCは非常に早期から食道静脈瘤ができやすいということを知っておく必要があると思います．

西原　抗ミトコンドリア抗体がPBCの標識自己抗体として使いやすいので，原因の分からない肝臓病があって，胆道系酵素の値が高い場合には，一度チェックしていただきたいと思います．

従来は肝硬変になって見つかっていたPBCが，現在では無症候性の段階で8割方見つかりますので，食道静脈瘤に留意する必要のあ

*2平成16年度の診断基準の改定により，食道静脈瘤のみを有する場合も症候性PBCに分類されることになった．