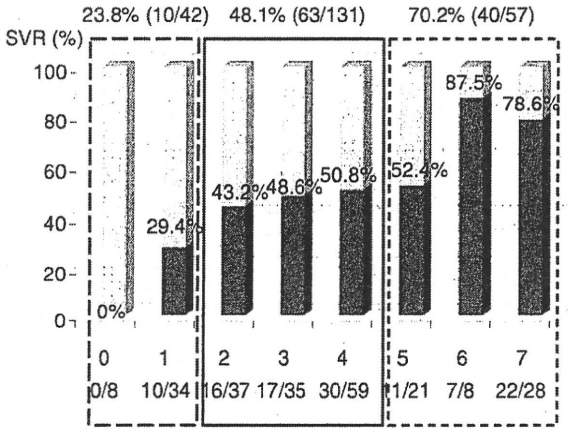

Figure 1 Scoring data according to formula A and the SVR rate in the 230 patients with chronic hepatitis C with high viral loads and treated with PEG-IFN and RBV combination therapy (Group A). Patients were classified into a poorly responsive group (score 0 to 1), a moderately responsive group (score 2 to 4) and a moderately to highly responsive group (score 5 to 7).

RNA load at baseline was 0.002 with the highest Odds ratio (2.579), we set point 3 to HCV RNA load <1000 KIU. Similarly, because the *P*-value of gender was 0.004 with higher Odds ratio (2.277), we set point 2 to male gender. The *P*-values of platelet counts and age were not statistically significant. However, because the Odds ratios of these two items were relatively high (1.624 and 1.510), we set point 1 to platelet counts $\geq 15 \times 10^4$ /mm^3 and age <60. Based on these data, a simple formula was constructed: male gender (point 2) + HCV RNA load <1000 KIU (point 3) + platelet counts $\geq 15 \times 10^4$ /mm^3 (point 1) + age <60 (point 1). This formula was referred to as formula A.

For easy use of formula A in clinical practice, patients in group A could be classified into three groups depending on their response to therapy, that is, poorly responsive (point 0 to 1), moderately responsive (point 2 to 4) and moderately to highly responsive (point 5 to 7) groups (Fig. 1). The SVR rate in the poorly responsive group was 23.8% (10/42), that in moderately responsive group was 48.1% (63/131) and that in moderately to highly responsive group was 70.2% (40/57). To determine the efficacy of formula A, we applied it to group B (Fig. 2). The poorly responsive group (point 0 to 1) showed an SVR rate of 7.1% (1/14), the moderately responsive group (point 2 to 4) 38.6% (22/57) and the moderately to highly responsive group (point 5 to 7) 70.3% (26/37).

Impact of information on amino acid sequences in the ISDR and HCV core on the accuracy of formula A

Because amino acid mutations in the ISDR and substitutions in core region of HCV affect the responsiveness to Peg-IFN/RBV combination therapy,[8-10] we constructed another formula by adding this information, but without liver histology. Because patients with ≥2 amino acid mutations in the ISDR and HCV core amino acid 70 wild type have higher probability to attain SVR,[8-10] we performed multivariate logistic regression analysis with six items (gender, HCV RNA load at baseline, platelet counts, age, amino acid substitutions in ISDR and HCV core amino acid 70) and the *P*-values were calculated to be 0.009, 0.008, 0.143, 0.204, 0.051 and 0.023, respectively (Table 3).

Because the *P*-values of gender and HCV RNA load at baseline were 0.009 and 0.008 with high Odds ratios (3.357 and 3.471), we set point 3 to male gender and HCV RNA load at baseline <1000 KIU. Similarly, because the *P*-values of ISDR mutation and Core 70 mutant/wild type were 0.051 and 0.023 with relatively high Odds ratios (2976 and 3.139), we set point 2 to ≥2 amino acid substitutions in ISDR and HCV core amino acid 70 wild type. The *P*-values of platelet counts and age were not statistically significant. However, because the Odds ratios of these two items were relatively high (2.021 and

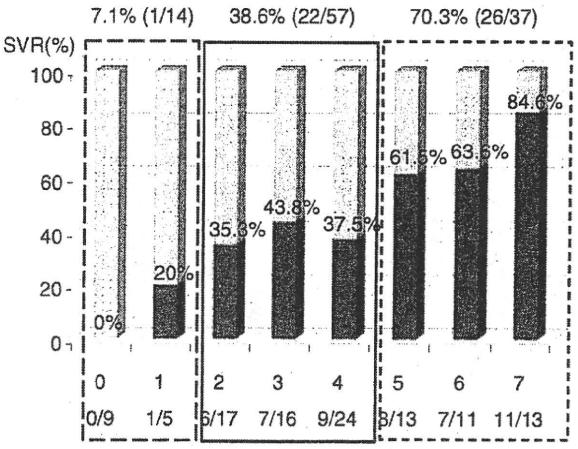

Figure 2 Scoring data according to formula A and the SVR rate in the 108 patients with chronic hepatitis C with high viral loads and treated with PEG-IFN and RBV combination therapy (Group B). Score 0 to 1 represents a poorly responsive group, score 2 to 4 a moderately responsive group and score 5 to 7 a moderately to highly responsive group, which is similar to the data presented in Figure 1.

Table 3 Multivariate logistic regression analysis, based on SVR and non-SVR in the 108 patients with chronic hepatitis C with high viral loads and treated with PEG-IFN and RBV combination therapy (Group B). Based on this result, formula B was constructed

	Odds ratio	(95% CI)	*P* value
Gender (female/male)	3.357	(1.346–8.375)	0.009
HCV RNA			
(1000 KIU/mL ≥/<)	3.471	(1.390–8.666)	0.008
PLT ($15 \times 10^4/\mu L$ ≥/<)	2.021	(0.895–2.944)	0.143
Age (60 years old ≥/<)	1.929	(0.700–5.316)	0.204
ISDR mutation (0.1/≥2)	2.976	(0.995–8.904)	0.051
Core 70 mutant/wild type	3.139	(1.172–8.406)	0.023

1.929), we set point 1 to platelet counts $\geq 15 \times 10^4$ /mm^3 and age <60. Based on these data, formula B was constructed: male gender (point 3) + HCV RNA load at baseline <1000 KIU (point 3) + platelet counts $\geq 15 \times 10^4$ /mm^3 (point 1) + age <60 (point 1) + ≥2 amino acid substitutions in ISDR (point 2) + HCV core amino acid 70 wild type (point 2). In group B, a total score of 0 to 3 could be categorized as the poorly responsive group (SVR ratio: 4.8% [1/21]), that of 4 to 7 the moderately responsive group (SVR ratio: 43.6% [27/62]) and that of 8 to 12 the moderately to highly responsive group (SVR ratio: 84% [21/25]) (Fig. 3).

DISCUSSION

IN THIS STUDY, we constructed a formula to predict the efficacy of Peg-IFN/RBV combination therapy:

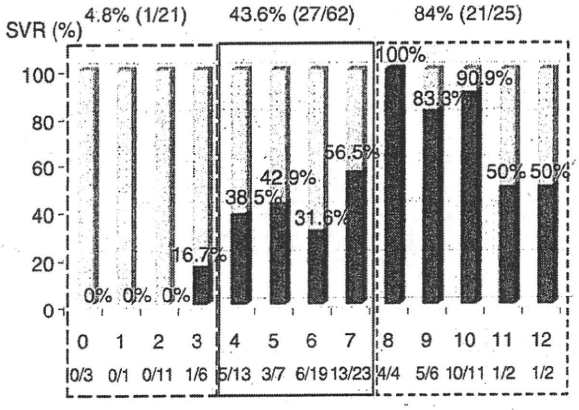

Figure 3 Scoring data according to formula B and the SVR rate in the 108 patients with chronic hepatitis C with high viral loads and treated with PEG-IFN and RBV combination therapy (Group B). Score 0 to 3 represents a poorly responsive group, score 4 to 7 a moderately responsive group and score 8 to 12 a highly responsive group.

male gender (point 2) + HCV RNA load at baseline <1000 KIU (point 3) + platelet counts $\geq 15 \times 10^4$ /mm^3 (point 1) + age <60 (1 point). This simple formula (formula A) could distinguish a poorly responsive group (score [0–1]), a moderately responsive group (score [2–4]) and a moderately to highly responsive group (score [5–7]) (Fig. 1). Thus, formula A may be used by general physicians easily to roughly guess the probability of response to Peg-IFN and RBV combination therapy at the patient's first visit. Another formula (formula B) was constructed by adding the information of amino acid substitutions in the HCV genome. Although examination of amino acid substitutions in the HCV genome is not covered by the public health insurance in Japan, formula B distinguished a poorly responsive group (score [0–3]), a moderately responsive group (score [4–7]) and a highly responsive group (score [8–12]) (Fig. 3).

In Peg-IFN and RBV combination therapy for CH-C with a high viral load, the interval between the start of therapy and disappearance of HCV RNA from the serum is widely accepted as the most reliable marker to predict outcome,[12] and response-guided therapy is recommended. According to nationwide registration trials in Japan, in patients with a rapid virological response (RVR), demonstrating disappearance of HCV RNA within the first four weeks, the SVR rate was expected to be 76% to 100%, and in patients with an early virological response (EVR), showing the disappearance of HCV RNA in the first 5 to 12 weeks, the SVR rate was expected to be 71% to 73%.[13,14] In contrast, in patients with a late virological response (LVR), demonstrating clearance of HCV RNA between weeks 13 to 24, the expected SVR rate was as low as 29 to 36%. However, in clinical practice, most patients are happy to know the probability of SVR at the first or second visit, or at least before starting therapy. In this regard, formula A we advocate may be useful for a wide range of physicians.

According to formula B which included the substitutions of amino acids in the ISDR and HCV core, the predicted SVR rate also was classified into three groups, and with increased accuracy (Fig. 3). Recently, a strong association between interleukin 28B (IL28B) gene polymorphism and the response to PEG-IFN and RBV combination therapy was reported for CH-C patients.[15–17] Because determination of IL28B gene polymorphism as well as the amino acid sequences of the ISDR or HCV core is not covered by the public health insurance in Japan, it is difficult to advocate a formula containing these factors for a wide range of Japanese general physicians.

In patients with CH-C, liver biopsy is recommended to determine the treatment.[12] Because liver biopsy is not required for IFN-based antiviral therapy in Japanese public health insurance, a proportion of the patients refuse liver biopsy but are willing to be treated by Peg-IFN and RBV combination therapy. In this regard, formula A is useful in providing information concerning the likely efficacy of treatment at the first or second visit.

We constructed a simple formula to predict the outcome of treatment of genotype 1 CH-C with high viral load with Peg-IFN and RBV for 48 weeks. Recently, response-guided therapy recommended prolonged therapy up to 72 weeks for patients with LVR.[18–21] A larger study is required to establish a better formula to be utilized readily by the general physicians.

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REFERENCES

1 Manns MP, McHutchinson JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet* 2001; **358**: 958–65.
2 Fried MW, Schiffman ML, Reddy KR *et al.* Peginterferon alfa2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975–82.
3 Chung H, Ueda T, Kudo M. Changing trends in hepatitis C infection over the past 50 years in Japan. *Intervirology* 2010; **53**: 39–43.
4 Izumi N, Nishiguchi S, Hino K *et al.* Management of hepatitis C: Consensus of Japan Society of Hepatology. *Hepatol Res* 2010; **40**: 347–68.
5 Iwasaki Y, Ikeda H, Araki Y *et al.* Limitations of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* 2006; **43**: 54–63.
6 Shirakawa H, Matsumoto A, Joshita S *et al.* Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. *Hepatology* 2008; **48**: 1753–60.
7 Saito H, Ebinuma H, Ojiro K *et al.* On-treatment predictions of success in peginterferon/ribavirin treatment using a novel formula. *World J Gastroenterol* 2010; **16**: 89–97.
8 Akuta N, Suzuki F, Sezaki H *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response in interferon-ribavirin combination therapy. *Intervirology* 2005; **48**: 372–80.
9 Akuta N, Suzuki F, Kawamura Y *et al.* Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1a: amino acid substitutions in the core region and low-density lipoprotein cholesterol level. *J Hepatol* 2007; **46**: 403–10.
10 Enomoto N, Sakuma I, Asahina Y *et al.* Mutations in the nonstructural protein 5 A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; **334**: 77–81.
11 Kato N, Hijikata M, Ootsuyama Y *et al.* Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. *Proc Natl Acad Sci U S A* 1990; **87**: 9524–8.
12 Izumi N, Nishiguchi S, Hino K *et al.* Management of Hepatitis C: Consensus of Japan Society of Hepatology 2010. *Hepatol Res* 2010; **40**: 347–68.
13 Iino S, Okita K, Omata M *et al.* Clinical efficacy of PEG-Interferon alfa-2b and ribavirin combination therapy for 48 weeks in chronic hepatitis C patients with genotype 1 and high viral load –retrospective comparison with Interferon alfa-2b and ribavirin combination therapy for 24 weeks. *Kantansui* 2004; **49**: 1099–121.
14 Yamada G, Iino S, Okuno T *et al.* Virological response in patients with hepatitis C virus genotype 1b and a high viral load: impact of peginterferon- alpha-2a plus ribavirin dose reductions and host-related factors. *Clin Drug Investig* 2008; **28**: 9–16.
15 Ge D, Fellay J, Thompson AJ *et al.* Genetic variation in IL28B predict hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399–401.
16 Tanaka Y, Nishida N, Sugiyama M *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105-11-9.
17 Suppiah V, Moldovan M, Ahlenstiel G *et al.* IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100–4.
18 Berg T, von Wagner M, Nasser S *et al.* Extended treatment duration for hepatitis C virus type 1: comparing 48 versus

72 weeks of peginterferon-alfa-2a plus ribavirin. *Gastroenterology* 2006; **130**: 1086–97.
19 Sanchez-Tapies JM, Diago M, Escartin P *et al.* Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; **131**: 451–60.
20 Pearlman BL, Ehleben C, Saifee S *et al.* Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis C genotype 1-infected slow responders. *Hepatology* 2007; **46**: 1688–94.
21 Ide T, Hino T, Ogata K *et al.* A randomized study of extended treatment with peginterferon alpha-2b plus ribavirin based on time to HCV RNA negative-status in patients with genotype 1b chronic hepatitis C. *Am J Gastroenterol* 2009; **104**: 70–5.

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Reduced risk of hepatocellular carcinoma after interferon therapy in aged patients with chronic hepatitis C is limited to sustained virological responders

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SUMMARY. This study was undertaken to investigate the effect of interferon (IFN) monotherapy on the risk of hepatocellular carcinoma (HCC) in aged-patients with chronic hepatitis C. Seven hundred and twenty-five patients with histologically proven chronic hepatitis C were enrolled in this retrospective cohort study; 531 received IFN monotherapy for 6 months between 1992 and 1995, and 157 were collected as a historical control. The effect of IFN therapy on the development of HCC was compared between the patients with chronic hepatitis C under 60 years old (non-aged group, $n = 531$) and those 60 and over (aged group, $n = 194$). A stepwise Cox proportional-hazards regression analysis in the non-aged group revealed that IFN therapy (risk ratio 0.52, 95% CI 0.33–0.81, $P = 0.004$), older age ($P = 0.001$), and higher histological stage ($P < 0.001$) were independent factors associated with the development of HCC. In the aged-group, only higher histological stage ($P = 0.002$) and male gender ($P = 0.011$), but not IFN therapy (risk ratio 0.77, 95% CI 0.42–1.40, $P = 0.386$), were identified as independent risk factors for HCC, although HCC was significantly reduced when sustained virological response (SVR) was obtained (risk ratio 0.23, 95% CI 0.08–0.64, $P = 0.005$). In conclusion, inhibitory effect of IFN on development of HCC in the patients with chronic hepatitis C aged 60 and over was limited to the patients achieving SVR when treated with 6 months-IFN monotherapy.

Keywords: aged patients, chronic hepatitis C, hepatocellular carcinoma, interferon, sustained virological response.

INTRODUCTION

In Japan, based on the epidemiological surveillance as well as the study on molecular tracing of hepatitis C virus (HCV), HCV infection is considered to spread from the 1920s and to expand more after World War II [1–5], The data of first-time blood donor candidates in Osaka demonstrated that the prevalence of anti-HCV antibodies among the candidates born in 1925–1935 was 7–10%, which was much higher than the prevalence of anti-HCV antibodies among the younger population [6]. Accordingly, chronic hepatitis C patients have become aged in Japan and HCV-related hepatocellular carcinoma (HCC) patients have also been shown to be old with a peak around age 70 and tended to decrease [1,3,5]. More importantly, the main cause of death in the patients with chronic hepatitis C has been reported to be HCC [7–10].

In the 1990s, interferon (IFN) therapy was used for the treatment of the patients with chronic hepatitis C worldwide and it has been shown by many studies including our reports that IFN therapy reduced the risk of HCC in patients with chronic hepatitis C [7,11–17]. This inhibitory effect of IFN therapy on hepatocarcinogenesis is notable when sustained virological response (SVR) was obtained, although SVR rate of IFN monotherapy was not very high. It has been also

Abbreviations: IFN, interferon; HCC, hepatocellular carcinoma; SVR, sustained virological response; HCV, hepatitis C virus; non-SVR, nonsustained virological response.

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reported that HCC development was significantly reduced in the patient achieving SVR as compared with those without SVR in chronic hepatitis C patients treated with IFN and ribavirin [18].

For the treatment of the patients with chronic hepatitis C, a combination of peginterferon and ribavirin has become a standard therapy, which has a high SVR rate [19–21]. However, the combination treatment has several adverse effects such as haemolytic anaemia which may not be tolerable for aged patients with chronic hepatitis C. On the other hand, aging is a significant risk factor for HCC in chronic hepatitis C patients. Accordingly, it is an important issue whether IFN monotherapy could reduce incidence of HCC in aged patients with chronic hepatitis C. Recently, Arase *et al.* [22] reported that long-term IFN monotherapy using low-dose of natural IFN-α was effective in preventing hepatocarcinogenesis in aged patients with chronic hepatitis C. In contrast, the hepatitis C antiviral long-term treatment against cirrhosis (HALT-C) Trial has shown that maintenance peginterferon therapy for 3.5 years did not reduce the incidence of HCC and the rate of disease progression in chronic hepatitis C patients with bridging fibrosis or cirrhosis who failed to respond to the combination therapy of peginterfeon-α2a and ribavirin [23,24].

We conducted a long-term multicenter retrospective cohort study to clarify the effect of 6-month IFN monotherapy on the incidence of HCC in aged patients with chronic hepatitis C.

MATERIAL AND METHODS

Patients

This study was conducted at Osaka University Hospital and six university-affiliated hospitals. IFN-treated patients consisted of 568 consecutive patients with chronic hepatitis C who had undergone liver biopsy 1 week to 2 months before IFN therapy and received either human lymphoblastoid IFN, recombinant IFN-α2a or recombinant IFN-α2b for 6 months between 1992 and 1995. The control group consisted of 158 consecutive patients with chronic hepatitis or cirrhosis who had undergone liver biopsy between January 1986 and December 1989, when IFN therapy had not been available in Japan. All the patients were positive for anti-HCV. The inclusion criteria in this study were as follows: (1) histological diagnosis of chronic hepatitis or cirrhosis; (2) no history of clinical signs at entry into the study of complications of cirrhosis, i.e. ascites, jaundice, encephalopathy, or variceal bleeding; (3) no previous IFN therapy; (4) no evidence of HCC at entry into the study as assessed by ultrasonography and/or computed tomography; (5) absence of serum hepatitis B surface antigen; (6) absence of co-existing liver diseases such as autoimmune hepatitis or primary biliary cirrhosis and (7) absence of excessive alcohol consumption (>80 g/day).

Sustained virological response was defined as persistent HCV RNA negativity during IFN therapy and follow-up. Patients showing positive HCV RNA after IFN therapy were classified as nonsustained virological response (non-SVR). In the patients with non-SVR, patients whose ALT levels decreased to the normal range and remained normal during IFN therapy were classified as transient biochemical response and patients without a decrease of ALT levels of the normal range during the therapy were classified as zbiochemical nonresponse.

Hepatitis C virus antibody was measured by first-, second-, or third-generation enzyme-linked immunosorbent assays (Ortho Diagnostics, Tokyo, Japan). Serum HCV RNA was measured by reverse transcription polymerase chain reaction or complementary DNA assay [25].

Follow-up

The starting date of follow-up of the patients was defined as the date of liver biopsy. Abdominal ultrasonography or computed tomography and biochemical examinations including α-fetoprotein were performed every 3–6 months during follow-up equally in the IFN-treated and control patients. The diagnosis of HCC was confirmed by needle biopsy, by surgically resected tumour specimens, or by typical radiological findings on hepatic angiography or dynamic computed tomography. In the patients residing in Osaka whose follow-up data were not obtained, the Osaka Cancer Registry was used to determine whether HCC had occurred and the data were available until the end of 2002 in this study [13,26]. Accordingly, we decided to use the date of the development of HCC or the end of 2002 as the end of follow-up. As the longest observation period of the patients in the IFN group was 11 years, only the follow-up data for the first 11 years were considered in the control group. The study protocol was in accordance with the Helsinki Declaration of 1975 (revised in 1983) and approved by the Ethical Committee of the Ikeda Municipal Hospital.

Histological evaluation

The sections were stained with haematoxylin–eosin and Azan-Mallory and histology of liver biopsy specimens was scored by two authors in a blinded manner using two scoring methods as described before [13]. Briefly, fibrosis score of Desmet *et al.* was used for the assessment of histological staging and a total score of histological activity (components 1–3) using the Knodell histological activity index was used for the assessment of histological grading [13,27,28].

Statistical analysis

Patients who did not complete the treatment protocol were included for the analysis on an intention-to-treat basis. The chi-square test and Student's *t*-test were used to compare the

baseline characteristics. The Kaplan–Meier method was used to calculate the cumulative incidence of HCC, and the log-rank test was used to compare the cumulative incidence of HCC between the groups. To estimate independent risk factors for the development of HCC, a stepwise Cox proportional-hazards regression analysis was used. For the analysis, IFN therapy, age, gender, and histological staging and activity scores were used as variables. A P value <0.05 was considered statistically significant. Data are presented as the mean ± SD and were analysed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1 shows the baseline characteristics of the aged (60 years old and over) and non-aged (under 60 years old) groups. Both the histological stage and activity were significantly higher in the aged group than in the non-aged group. The proportion of male patients of the non-aged group was significantly higher than that of the aged group. In Table 2, baseline characteristics of controls and IFN-treated patients in the aged and non-age groups were compared. In the non-aged group, age at entry, proportion of male gender, histological activity score, serum ALT level and platelet count did not differ between the control and IFN-treated patients. However, histological stage of IFN-treated patients was less advanced as compared with that of the control patients. In the age-group, age at entry, proportion of male gender, histological stage and activity, serum ALT level and platelet count did not differ between the control and IFN-treated patients.

During the follow-up period, HCC was found in 35 controls and 44 IFN-treated patients among the non-aged group and in 14 controls and 48 IFN-treated patients among the aged group. The median tumour sizes of HCC in controls and IFN-treated patients at the time of discovery on ultrasonography or computed tomography were 22 mm (range, 10–55 mm) and 19 mm (range, 8–52 mm) respectively ($P \geq 0.2$). In the non-aged group, the cumulative incidence of HCC estimated by the Kaplan–Meier Method of IFN-treated patients was significantly lower than that of control patients (log-rank test, $P < 0.001$, Fig. 1a), whereas there was no difference in the cumulative incidence of HCC between controls and IFN-treated patients in the aged group (log-rank test, $P = 0.498$, Fig. 1b). The cumulative incidence of HCC of SVR and non-SVR patients and controls of the aged and non-aged groups are shown in Fig. 2. The 10-year incidences of HCC for controls, non-SVR and SVR patients in the non-aged group were 30.1%, 15.8%, 4.5% respectively (log-rank test, $P < 0.001$, Fig. 2a). Also, the 10-year incidences of HCC for controls, non-SVR and SVR patients in the aged group were 39.1%, 38.9%, 12.7% respectively (log-rank test, $P = 0.015$, Fig. 2b).

In Table 3, risk ratios for the development of HCC calculated by a stepwise Cox regression analysis in the aged and non-aged patients with chronic hepatitis C according to virological and biochemical responses to IFN are summarized. In the 410 IFN-treated patients of non-aged group, 134 patients (32.7%) achieved SVR and the remaining 276 showed non-SVR (Table 3). Of this 276 patients showing non-SVR, 163 showed transient biochemical response and 113 showed biochemical nonresponse during the IFN treatment. On the other hand, 41 (25.9%) of 158 IFN-treated patients of the aged group obtained SVR and the other 117 did not obtain SVR (Table 3). Of the 117 non-SVR patients, 57 showed transient biochemical response and 60

Table 1 Baseline characteristics of aged and non-aged patients with chronic hepatitis C

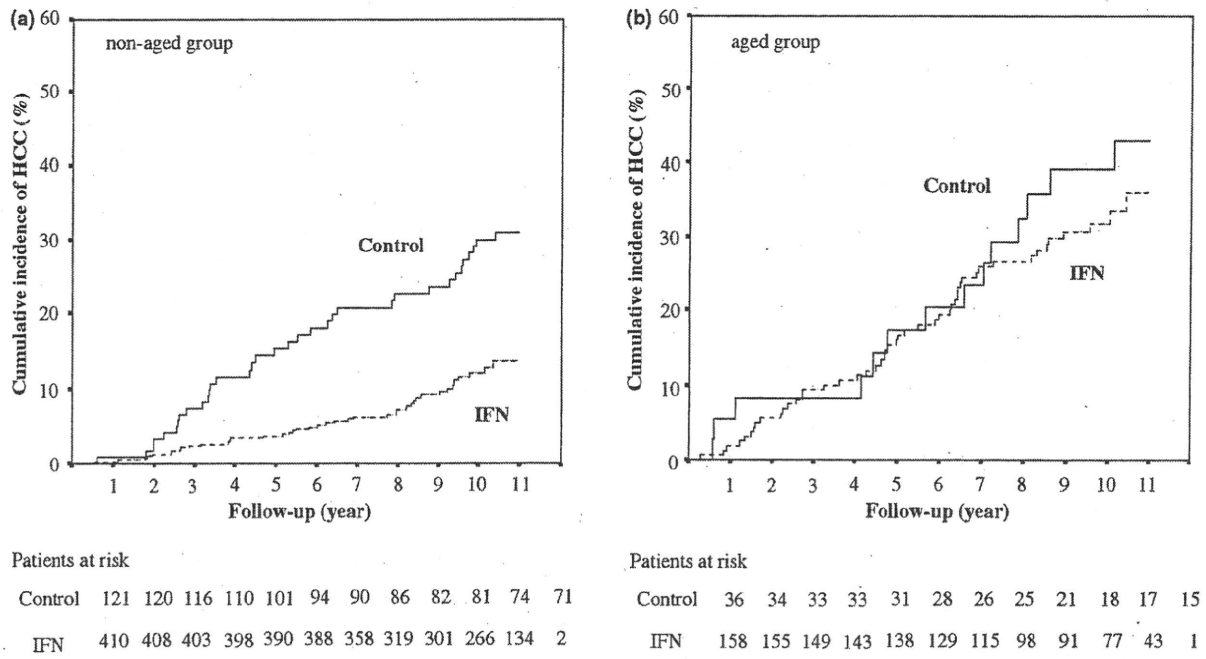
	Non-aged group (n = 531)	Aged group (n = 194)	P value
Control group (n)/IFN group (n)	121/410	36/158	0.262
Age	48.1 ± 9.7	63.7 ± 3.3	<0.001
Gender			
Male	353	108	0.009
Female	178	86	
Histological stage*			
F0, 1	186	37	0.001
F2	157	69	
F3	141	69	
F4	47	19	
Histological activity†			
<10	329	104	0.049
≧10	202	90	
ALT (IU/L)	117 ± 86	104 ± 60	0.053
Platelete count ($10^4/\mu$L)	15.4 ± 5.6	14.4 ± 5.6	0.040

*According to Desmet *et al.*[27] †Based on components 1–3 of the Knodell histological activity.

Table 2 Baseline characteristics of controls and IFN-treated patients in aged and non-aged groups

	Non-aged group			Aged group		
	Controls	IFN-treated	*P* value	Controls	IFN-treated	*P* value
n	121	410		36	158	
Age	48.4 ± 10.5	48.0 ± 9.4	0.736	64.6 ± 3.6	63.5 ± 3.2	0.059
Gender						
Male	75	278	0.273	22	86	0.579
Female	46	86		14	72	
Histologic stage*						
F0,1	27	159	<0.001	8	29	0.933
F2	28	129		12	57	
F3	47	94		12	57	
F4	19	28		4	15	
Histologic activity†						
<10	72	257	0.525	20	84	0.854
≧10	49	153		16	74	
ALT (IU/L)	127 ± 80	114 ± 88	0.132	110 ± 85	103 ± 53	0.523
Platelete count ($10^4/\mu L$)	15.2 ± 6.1	15.4 ± 5.4	0.766	15.0 ± 5.4	14.3 ± 5.7	0.486
HCV RNA load						
High	ND‡	166		ND‡	54	
Low	ND‡	116		ND‡	30	
HCV RNA serotype						
1	ND‡	231		ND‡	90	
2	ND‡	102		ND‡	32	

*According to Desmet *et al.* [27] †Based on components 1–3 of the Knodell histologic activity. ‡Not done.



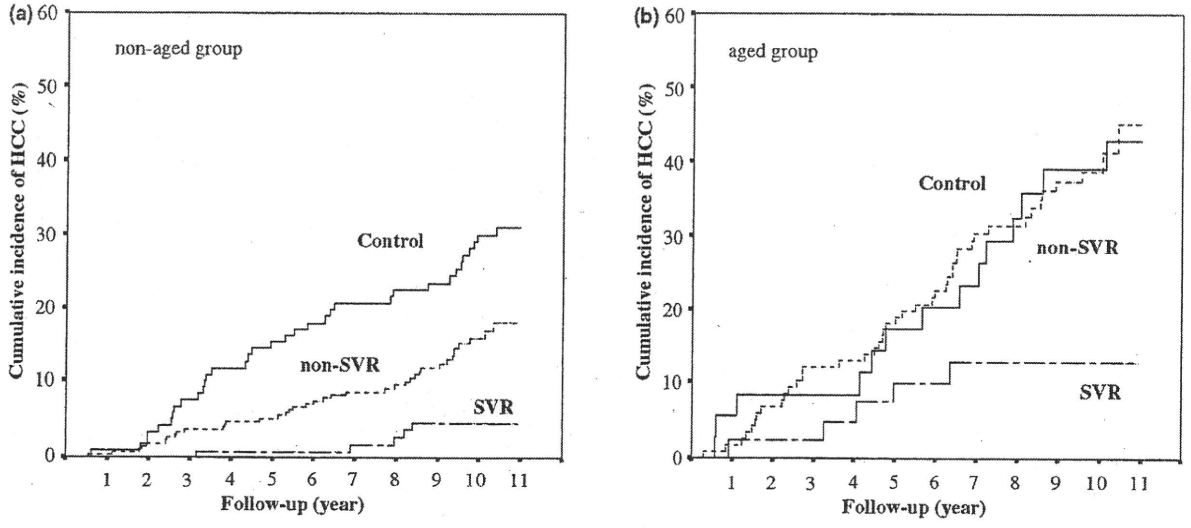
Patients at risk

Control	121	120	116	110	101	94	90	86	82	81	74	71
IFN	410	408	403	398	390	388	358	319	301	266	134	2

Patients at risk

Control	36	34	33	33	31	28	26	25	21	18	17	15
IFN	158	155	149	143	138	129	115	98	91	77	43	1

Fig. 1 Cumulative incidence of hepatocellular carcinoma in IFN-treated (dotted line) and control (solid line) patients of the non-aged group (a) and the aged group (b). A log-rank test of the two curves showed a significant difference in the non-aged group ($P < 0.001$), whereas no significant difference was observed in the aged group ($P = 0.498$).



Patients at risk

Control	121	120	116	110	101	94	90	86	82	81	74	71
Non-SVR	276	274	269	264	259	257	239	212	199	179	85	2
SVR	134	134	134	134	131	131	119	107	102	87	49	0

Patients at risk

Control	36	34	33	33	31	28	26	25	21	18	17	15
Non-SVR	117	115	115	103	100	93	82	67	60	50	26	1
SVR	41	40	40	40	38	36	33	31	31	27	17	0

Fig. 2 (a) Cumulative incidence of hepatocellular carcinoma categorized by sustained virological response (dashed line), nonsustained virological response (dotted line), and controls (solid line) of the non-aged group (a) and the aged group (b). A log-rank test of the three curves showed a significant difference between these groups (non-aged group, $P < 0.001$; aged group, $P = 0.015$).

showed biochemical nonresponse. In the non-aged group, stepwise Cox regression analysis identified IFN therapy (risk ratio 0.52, 95% CI 0.33–0.81, $P = 0.004$), older age (risk ratio 1.07, 95% CI 1.03–1.10, $P = 0.001$), and higher histological stage (score 3 or 4) (risk ratio 4.03, 95% CI 2.41–6.76, $P < 0.001$) as independent risk factors associated with the development of HCC. In the non-aged group, the development of HCC was strongly suppressed when SVR was achieved (risk ratio 0.20, 95% CI 0.08–0.50, $P < 0.001$) (Table 3). In the patients with transient biochemical response of the non-SVR group among the non-aged group, HCC development was also significantly reduced (risk ratio 0.47, 95% CI 0.26–0.86, $P = 0.015$). In the aged group, stepwise Cox regression analysis revealed that only higher histological stage (score 3 or 4) (risk ratio 2.27, 95% CI 1.36–3.78, $P = 0.002$) and male gender (risk ratio 2.00, 95% CI 1.17–3.41, $P = 0.011$) were independent factors responsible for the development of HCC (Table 3). Although IFN therapy was not identified as an independent variable for HCC, the risk of HCC was significantly decreased in the patients with SVR in the aged group as shown in the Table 3 (risk ratio 0.23, 95% CI 0.08–0.64, $P = 0.005$). In the

Table 3 Risk ratios for hepatocellular carcinoma in aged and non-aged patients with chronic hepatitis C according to virological and biochemical responses to interferon*

	Non-aged group (n = 531)				Aged group (n = 194)			
	n	Risk ratio	95% CI	P value	n	Risk ratio	95% CI	P value
Control group	121	1.00			36	1.00		
IFN group	410	0.52	0.33–0.81	0.004	158	0.77	0.42–1.40	0.388
Sustained virological response	134	0.20	0.08–0.50	0.001	41	0.23	0.08–0.64	0.005
Nonsustained virological response	276	0.65	0.41–1.03	0.068	117	1.07	0.58–1.97	0.821
Transient biochemical response†	163	0.47	0.26–0.86	0.015	57	0.67	0.32–1.43	0.303
Biochemical nonresponse†	113	0.86	0.51–1.47	0.584	60	1.46	0.77–2.78	0.245

*A stepwise Cox regression analysis was carried out by using interferon therapy, age, gender, and histologic stage and histologic activity scores as variables. †Nonsustained virological response was classified into transient biochemical response and biochemical nonresponse according to the ALT response during the interferon treatment.

patients with transient biochemical response of the non-SVR group of aged patients, HCC development was not reduced (risk ratio 0.67, 95% CI 0.32–1.43, $P = 0.303$, Table 3) in contrast to the patients showing transient biochemical response in the non-aged group.

As the cumulative incidence of HCC calculated by the Kaplan–Meier Method of the patients with SVR in the aged group was much higher than that in the non-aged group, we also carried out Cox proportional-hazards regression analysis to estimate risk factors responsible for HCC development in the 175 patients achieving SVR. As a result, older age (risk ratio 1.09, 95% CI 1.01–1.18, $P = 0.025$) and higher histological activity before IFN therapy started (10 or more of the total score of components 1–3 in Knodell's histological activity index) (risk ratio 4.16, 95% CI 1.07–16.25, $P = 0.040$) were identified as risk factors associated with HCC among the patients with SVR.

DISCUSSION

In this long-term retrospective cohort study, an inhibitory effect of 6 months-IFN monotherapy in early 1990s on the cumulative incidence of HCC were compared between the patients with histologically proven chronic hepatitis C under 60 years old (non-aged group) and those 60 years old and over (aged group). Because of retrospective analysis, there were some differences in baseline characteristics between the two groups. In the aged group, the histological stage and activity as well as the proportion of male patients were significantly higher than in the non-aged group. Also, SVR rate in the aged group was lower than that in the non-aged group. To avoid the influence of these biases, we performed Cox proportional-hazards regression analysis to see whether IFN monotherapy reduced the risk of HCC in the aged and non-aged groups. Then, we found that IFN therapy for 6 months significantly reduced the risk of HCC (risk ratio 0.52) in the non-aged group, whereas this inhibitory effect of IFN monotherapy on HCC development was recognized only in the patients achieving SVR among the aged-patients.

It is difficult to explain why IFN had no inhibitory effect on HCC development in the aged patients, whereas IFN had significant inhibitory effect in the non-aged patients of this study. Many clinical studies have demonstrated that aging was an independent risk factor associated with HCV-related HCC other than advanced histological staging and male gender [7,11–17,29]. However, molecular mechanism of the impact of aging on haepetocarcinogenesis has not been elucidated. Moriya *et al.* reported that lipid hydroperoxide products accumulated in the liver without inflammation and may play a role in the development of HCC in HCV core gene transgenic mice [30,31]. A long-term infection of HCV may lead to HCC through some molecular alterations.

Recently, there have been two controversial reports from the United States and Japan as to the long-term effect of low-dose IFN therapy on the incidence of HCC in chronic hepatitis C [22,24]. The report from Japan was a non-randomized retrospective study and observed beneficial effect of long-term natural IFN-α therapy on hepatocarcinogenesis in aged chronic hepatitis C patients [22]. The HALT-C Trial from the United States, a large prospective randomized study, reported that treatment with peginterfeon-α2a at a dose of 90 μg weekly for 3.5 years did not prevent HCC development in the patients with bridging fibrosis or cirrhosis who did not obtain SVR by combination therapy of peginterferon and ribavirin [24]. The result was consistent with our data in the aged patients. However, the annual incidence of HCC of the HALT-C Trial, about 1%, was much lower than that in the aged group in this study, about 4%. Accordingly, a randomized prospective study to determine the effect of long-term IFN or peginterferon therapy on the incidence of HCC in chronic hepatitis C, especially in the aged patients, may be needed in Japan.

This study has a limitation, because we used historical controls as control patients. A lead-time bias may have occurred. Detection of HCC by the screening program could be less effective in controls than IFN-treated patients. In that case, we might underestimate the effect of IFN on the cumulative incidence of HCC. However, such underestimation may be unlikely as the tumour sizes at the time of detection were not different between the control and IFN-treated patients.

The 10-year incidence of HCC for SVR patients of the aged group (12.7%) was much higher than that of non-aged group (4.5%) in our study. Makiyama *et al.* [32] studied the risk factors for developing HCC after obtaining sustained biochemical response to IFN therapy in chronic hepatitis C and reported that older age, male gender and advanced fibrosis were associated with HCC. Consistent with their results, we found that older age was an independent risk factor for HCC in the patients with SVR, suggesting a high potential of developing HCC even after eradication of HCV RNA in the aged patients. Another possibility is that malignant foci, which could not be detected by imaging modalities, had already existed before IFN therapy. Our finding indicates that even in the patients showing SVR, a follow-up examination to investigate HCC should be carried out for at least 10 years, particularly in the aged patients.

In conclusion, IFN monotherapy reduced the risk of HCC in the patients with chronic hepatitis C under 60 years old. In contrast, this inhibitory effect of IFN on hepatocacinogenesis was limited to patients showing SVR in the aged-patients when treated with 6 months-IFN monotherapy. These results suggest that combination therapy of peginterferon and ribavirin is recommended even in the aged patients with chronic hepatitis C to obtain better preventive effect of IFN on HCC development. For reasons of relatively high cumulative incidence of HCC in the aged chronic hepatitis C patients with SVR to IFN therapy, they should be followed carefully even after eradication of HCV by IFN therapy.

REFERENCES

1 Yoshizawa H. Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. *Oncology* 2002; 62(Suppl. 1): 8–17.

2 Tsukuma H, Tanaka H, Ajiki W, Oshima A. Liver cancer and its prevention. *Asian Pac J Cancer Prev* 2005; 6: 44–50.

3 Tanaka H, Tsukuma H. Characteristics of Japanese patients with liver cancer – epidemiological study based on a comparison between male and female patients. *Hepatol Res* 2002; 24: S11–S20.

4 Tanaka Y, Kurbanov F, Mano S *et al.* Molecular tracing of the global hepatitis C virus epidemic predicts regional patterns of hepatocellular carcinoma mortality. *Gastroenterology* 2006; 130: 703–714.

5 Tanaka H, Imai Y, Hiramatsu N *et al.* Declining incidence of hepatocellular carcinoma in Osaka, Japan, from 1990 to 2003. *Ann Intern Med* 2008; 148: 820–826.

6 Tanaka H, Hiyama T, Tsukuma H *et al.* Prevalence of second generation antibody to hepatitis C virus among voluntary blood donors in Osaka, Japan. *Cancer Causes Control* 1994; 5: 409–413.

7 Tanaka H, Tsukuma H, Kasahara A *et al.* Effect of interferon therapy on the incidence of hepatocellular carcinoma and mortality of patients with chronic hepatitis C: a retrospective cohort study of 738 patients. *Int J Cancer* 2000; 87: 741–749.

8 Yoshida H, Arakawa Y, Sata M *et al.* Interferon therapy prolonged life expectancy among chronic hepatitis C patients. *Gastroenterology* 2002; 123: 483–491.

9 Kasahara A, Tanaka H, Okanoue T *et al.* Interferon treatment improves survival in chronic hepatitis C patients showing biochemical as well as virological responses by preventing liver-related death. *J Viral Hepat* 2004; 11: 148–156.

10 Imai Y, Kasahara A, Tanaka H *et al.* Interferon therapy for aged patients with chronic hepatitis C: improved survival in patients exhibiting a biochemical response. *J Gastroenterol* 2004; 39: 1069–1077.

11 Mazzella G, Accogli E, Sottili S *et al.* Alpha interferon treatment may prevent hepatocellular carcinoma in HCV-related liver cirrhosis. *J Hepatol* 1996; 24: 141–147.

12 Fattovich G, Giustina G, Degos F *et al.* Effectiveness of interferon alfa on incidence of hepatocellular carcinoma and decompensation in cirrhosis type C European Concerted Action on Viral Hepatitis (EUROHEP). *J Hepatol* 1997; 27: 201–205.

13 Imai Y, Kawata S, Tamura S *et al.* Relationship of interferon therapy and hepatocellular carcinoma in patients with chronic heaptitis C. *Ann Intern Med* 1998; 129: 94–99.

14 Nishiguchi S, Kuroki T, Nakatani S *et al.* Randomised trial of effects if interferon-α on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995; 346: 1051–1055.

15 Kasahara A, Hayashi N, Mochizuki K *et al.* Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. *Hepatology* 1998; 27: 1394–1402.

16 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. *Ann Intern Med* 1999; 131: 174–181.

17 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; 29: 1124–1130.

18 Kurokawa M, Hiramatsu N, Oze T *et al.* Effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with chronic hepatitis. *Hepatol Res* 2009; 39: 432–438.

19 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958–965.

20 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–982.

21 Hoofnagle JH, Seeff LB. Peginterferon and ribavirin for chronic hepatitis C. *N Engl J Med* 2006; 355: 2444–2451.

22 Arase Y, Ikeda K, Suzuki F *et al.* Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. *J Med Virol* 2007; 79: 1095–1102.

23 Di Bisceglie AM, Shiffman ML, Everson GT *et al.* Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* 2008; 359: 2429–2441.

24 Lok AS, Seeff LB, Morgan TR *et al.* Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009; 136: 138–148.

25 Kato N, Yokosuka O, Omata M, Hosoda K, Ohto M. Detection of hepatic C virus ribonucleic acid in the serum by amplification with polymers chain reaction. *J Clin Invest* 1990; 86: 1764–1767.

26 Murakami R, Tsukuma H, Ubukata T *et al.* Estimation of validity of mass screening program for gastric cancer in Osaka, Japan. *Cancer* 1990; 65: 1255–1260.

27 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: grading and staging. *Hepatology* 1994; 19: 1513–1520.

28 Knodell RG, Ishak KG, Black WC *et al.* Formulation and application of numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; 1: 431–435.

29 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993; 18: 47–53.

30 Moriya K, Fujie H, Shintani Y *et al.* The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nat Med* 1998; 4: 1065–1067.

31 Moriya K, Nakagawa K, Santa T *et al.* Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. *Cancer Res* 2001; 61: 4365–4370.

32 Makiyama A, Itoh Y, Kasahara A *et al.* Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after sustained response to interferon therapy. *Cancer* 2004; 101: 1616–1622.

C型肝炎の臨床最前線
肝胆膵増刊号

宿主細胞標的薬(Nitazoxanide/Cyclosporins)

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索引用語：Nitazoxanide，Alinia®，Cyclosporin A，Cyclophilin inhibitor，DEBIO-025

1 はじめに

C型慢性肝炎に対する抗ウイルス療法は，ペグインターフェロン(Pegylated interferon; Peg-IFN)/リバビリン(Ribavirin; RBV)併用療法が標準的な治療法となって，治療効果は飛躍的に向上した[1-3]．しかし，同療法においても，難治性であるgenotype 1型高ウイルス量症例では，約半数にHCV排除が得られず，さらなる治療効果向上が望まれる．

近年，新たなC型肝炎治療薬として，HCV選択的抗ウイルス剤である酵素阻害剤(プロテアーゼ阻害剤，ポリメラーゼ阻害剤)などの開発が進み，Peg-IFN/RBVに新規抗ウイルス薬を加えた3剤併用療法の臨床試験が進行中である．これら新規抗ウイルス薬との併用によりHCVに対する治療効果が増強する一方で，高度な血球異常の発現や，HCV変異ウイルスの出現も報告されている[4-7]．本邦のC型肝炎患者は高齢化が進んでおり，治療への忍容性が危惧されることから[8]，今後，治療効果のさらなる向上と合わせて，より安全性の高い薬剤の開発が求められている．

本稿では，酵素阻害剤と異なる作用機序でHCV増殖を抑制する新規抗ウイルス薬として，すでに海外において抗寄生虫薬として広く用いられているNitazoxanide(NTZ)と，各種臓器移植後の拒絶反応の抑制や自己免疫疾患の治療薬として広く用いられているCyclosporin A(CyA)やCyclosporinをもとに合成されたCyclophilin(Cyp)阻害剤であるDEBIO-025を中心に，最近の知見を紹介する．

2 Nitazoxanide

NTZは，寄生虫や細菌に対して広範囲にスペクトルを有するニトロチアゾールベンザミド誘導体である(図1)．海外では，ランブル鞭毛虫(*Giardia lamblia*)，またはクリプトスポリジウム(*Cryptosporidium parvum*)による下痢症の適応でFDAの承認を受け，商品名Alinia®として市販されている．

Norio HAYASHI et al: Efficacy of Nitazoxanide and cyclophilin inhibitor for chronic hepatitis C
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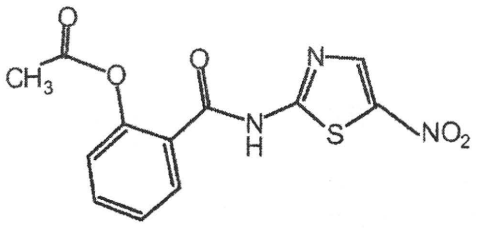

分子式：$C_{12}H_9N_3O_5S$，分子量：307.3

図1　Nitazoxanideの構造式

1. HCV治療薬としての開発・作用機序

NTZのC型慢性肝炎に対する開発は，クリプトスポリジウム症の治療を要したHIV/HCV重感染患者において，NTZ使用中にALTの改善傾向が認められたことから開始された．その後，NTZのHCV複製阻害作用について，HCVレプリコン細胞株を用いて検討した結果，NTZは，HCVレプリコンの複製を選択的かつ用量依存的に阻害し，この作用はIFNαやHCVポリメラーゼ阻害剤との併用により増強されることが確認された[9,10]．また，NTZによっても耐性ウイルスの出現を認めなかったことや，Telaprevir耐性ウイルスに対しても効果があることが確認されている[9]．

NTZのHCVに対する作用機序は，IFNと同様，細胞中の二重鎖RNAプロテインキナーゼ(PKR)系を活性化し，蛋白合成に必要な翻訳開始因子(eIF2α)をリン酸化して活性を失わせることにより，ウイルス由来の蛋白合成を阻害し，ウイルス増殖を抑制すると考えられている[11]．

2. C型慢性肝炎患者に対する臨床試験

C型慢性肝炎に対するNTZ単剤投与による第Ⅱ相試験[12]は，genotype 4のC型慢性肝炎47例を対象として，NTZ 500 mg×2/日群(23例)とplacebo群(24例)に分け，24週投与が行われた．この結果，投与終了時のHCV-RNA陰性化率(ETR)および著効率(SVR)は，placebo群でいずれも0％であったのに対し，NTZ群では，ETRが30％(7例)，SVRが17％(4例)で得られたと報告された．

NTZ単剤投与での結果を受けて，C型慢性肝炎に対するNTZとPeg-IFNα-2a/RBV併用療法の臨床試験が行われた．STEALTH C-1 study[13]は，初回治療のgenotype 4型C型慢性肝炎96例に対し，対照をPeg-IFNα-2a(180 μg/週)/RBV(1,000～1,200 mg/日)48週投与群(40例)として，NTZ(500 mg×2/日)12週間投与ののち，NTZ/Peg-IFNα-2a 2剤併用あるいはNTZ/Peg-IFNα-2a/RBV 3剤併用36週投与する群(それぞれ28例)の3群を比較する第Ⅱ相試験である．この結果，著効率は，対照群の50％に対し，NTZ 12週＋NTZ/Peg-IFNα-2a 36週投与で61％，NTZ 12週＋NTZ/Peg-IFNα-2a/RBV 36週投与で79％と，NTZ併用群において著効率が向上したと報告されている(図2)．発現頻度の高かった有害事象は，貧血，好中球減少および血小板減少であり，貧血の発現頻度がNTZ/Peg-IFNα-2a 2剤併用群において他のRBV併用群に比し低かった以外，有害事象の発現状況は各群同様であった．また，本試験と平行して，NTZの先行投与期間について比較する試験が行われ，初回治療のC型慢性肝炎44例(genotype 4：40例，genotype 1：3例，genotype 2：1例)を対象に，NTZ(500 mg×2/日)4週間投与ののち，NTZ/Peg-IFNα-2a 2剤併用36週投与を行った結果，SVRは80％と，NTZ 12週＋NTZ/Peg-IFNα-2a 36週投与と同等以上であったことが報告された[14]．

以上の結果を受けて，genotype 1型C型慢性肝炎に対する第Ⅱ相試験が行われた．初回投与のgenotype 1型C型肝炎112例を対象としたSTEALTH C-3 study[15]では，対照をplacebo 4週＋Peg-IFNα-2a(180 μg/週)/

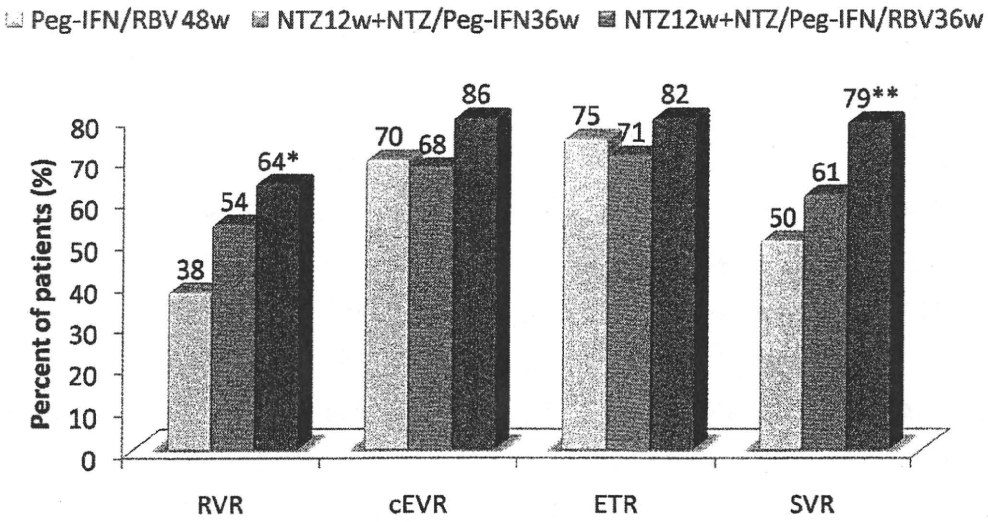

図2　初回投与のGenotype 4型C型慢性肝炎に対するNitazoxanide/Peg-IFNα-2a/Ribavirin併用療法の治療効果（文献13より改変）

RVR: rapid virologic response; 治療開始4週時のHCV-RNA陰性化
cEVR: complete early virologic response; 治療開始12週時のHCV-RNA陰性化
ETR: end-of-treatment virologic response; 治療終了時のHCV-RNA陰性化
SVR: sustained virologic response; 治療終了後24週時のHCV-RNA陰性化
*p=0.048; NTZ 12週＋NTZ/Peg-IFN/RBV 36週 vs. Peg-IFN/RBV 48週
**p=0.023; NTZ 12週＋NTZ/Peg-IFN/RBV 36週 vs. Peg-IFN/RBV 48週

RBV(1,000～1,200 mg/日)48週投与群(37例)として，NTZ(500 mg×2/日)4週＋NTZ/Peg-IFNα-2a/RBV 3剤併用48週投与(75例)を比較している．この中間報告では，治療開始16週および終了時点，治療終了後12週のHCV-RNA陰性化(＜50 IU/ml)が，対照群ではそれぞれ49％(18/37)，46％(17/37)，32％(12/37)であったのに対し，NTZ併用群では，それぞれ62％(45/73)，63％(46/73)，44％(32/73)と10％強の上乗せ効果があったことが報告されている(図3)．特に，高ウイルス例(＞800 KIU/mL)における治療終了後12週のHCV-RNA陰性化は，NTZ併用群で42％と対照群の29％に比し高率であった．一方，IFN既治療例に対するSTEALTH C-2 study[16]では，Peg-IFN/RBV併用療法で無効(治療開始12週でのHCV-RNA減少が2 $\log_{10}$未満，治療開始24週でのHCV-RNA陽性)であったgenotype 1型C型慢性肝炎64例を対象として，対照をplacebo 4週＋Peg-IFNα-2a(180 μg/週)/RBV(1,000～1,200 mg/日)48週投与群(22例)とし，NTZ(500 mg×2/日)4週＋NTZ/Peg-IFNα-2a/RBV 3剤併用48週投与(42例)を比較している．この最終報告では，対照群で治療中のHCV-RNA陰性化(＜50 IU/ml)および著効が1例も得られなかったのに対し，NTZ群では治療開始16週および終了時点のHCV-RNA陰性化が，それぞれ7％(3/42)，14％(6/42)，SVRが7％(3/42)で得られたと報告された．SVRが得られた3例は全例治療開始16週での陰性化例であり，うち2例は治療開始4週での陰性化例であったことから，治療早期での反応例については，治療効果が期待できる可能性があると考えられる．

NTZの安全性について，多くの症例での使用経験があることから，高齢化したC型慢性肝炎患者にも問題なく使用できることが期

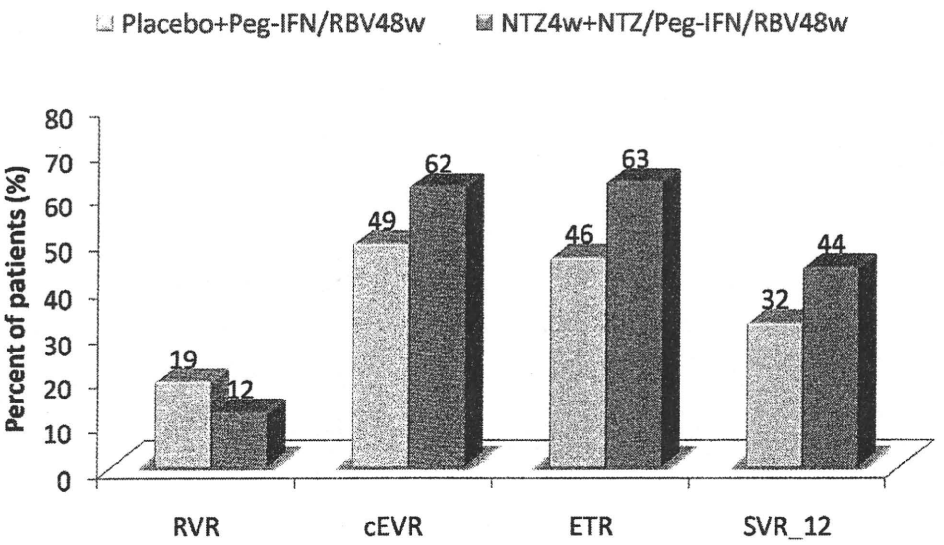

図3　初回投与のGenotype 1型C型慢性肝炎に対するNitazoxanide/Peg-IFNα-2a/Ribavirin併用療法の治療効果（文献15より改変）

RVR: rapid virologic response; 治療開始4週時のHCV-RNA陰性化
cEVR: complete early virologic response; 治療開始12週時のHCV-RNA陰性化
ETR: end-of-treatment virologic response; 治療終了時のHCV-RNA陰性化
SVR_12: sustained virologic response at 12 weeks after the treatment; 治療終了後12週時のHCV-RNA陰性化

待され，本邦における臨床試験が検討されている．

3 Cyclosporins

HCVウイルス蛋白そのものではなく，ウイルスと宿主蛋白の相互作用の研究から，宿主蛋白を標的とした薬剤の開発も行われており，Cyp阻害剤は，*in vitro*においてCyclosporinがHCV複製阻害作用を有するという知見により開発が開始された．宿主蛋白を標的とした治療法は，ウイルス蛋白を標的とした治療法に比べ，HCVウイルス変異を誘発しにくいという利点を有しており，またCyp阻害剤は，Cyclosporinの臨床使用経験があることから，比較的安全に投与できることが期待され，新たなHCV治療薬として注目を集めている．

1. HCV治療薬としての開発・作用機序

C型慢性肝炎患者において，IFNαとCyAを併用することにより，IFNα単独投与に比し有意に治療効果が向上することが報告された[17]．Cyclosporinの抗ウイルス効果について，HCV複製阻害作用がHCVレプリコン細胞株を用いて検討され，CyclosporinがHCVレプリコンの複製を用量依存的に阻害することが確認された[18,19]．一方，Cypは，Cyclosporinの細胞内受容体蛋白であり，蛋白質の高次構造の形成において重要な役割を担うPPIase（Peptidyl-prolyl cis-trans isomerase）活性を有しているが，CyclosporinはCypと結合し複合体を形成することでPPIase活性を阻害する．HCVにおいては，Cypの有するPPIase活性を阻害することにより，正常なHCV粒子の形成が阻害され，RNA複製が停止し，ウイルス増殖を抑制するという機序が想定されている[20,21]．DEBIO-025はCyclosporinをもとに合成された，免疫抑制作用のないCyp阻害剤であり，*in vitro*においてHCV増殖抑制効果が報告され[22]，*in vivo*でもHCVキメラマウスにおい

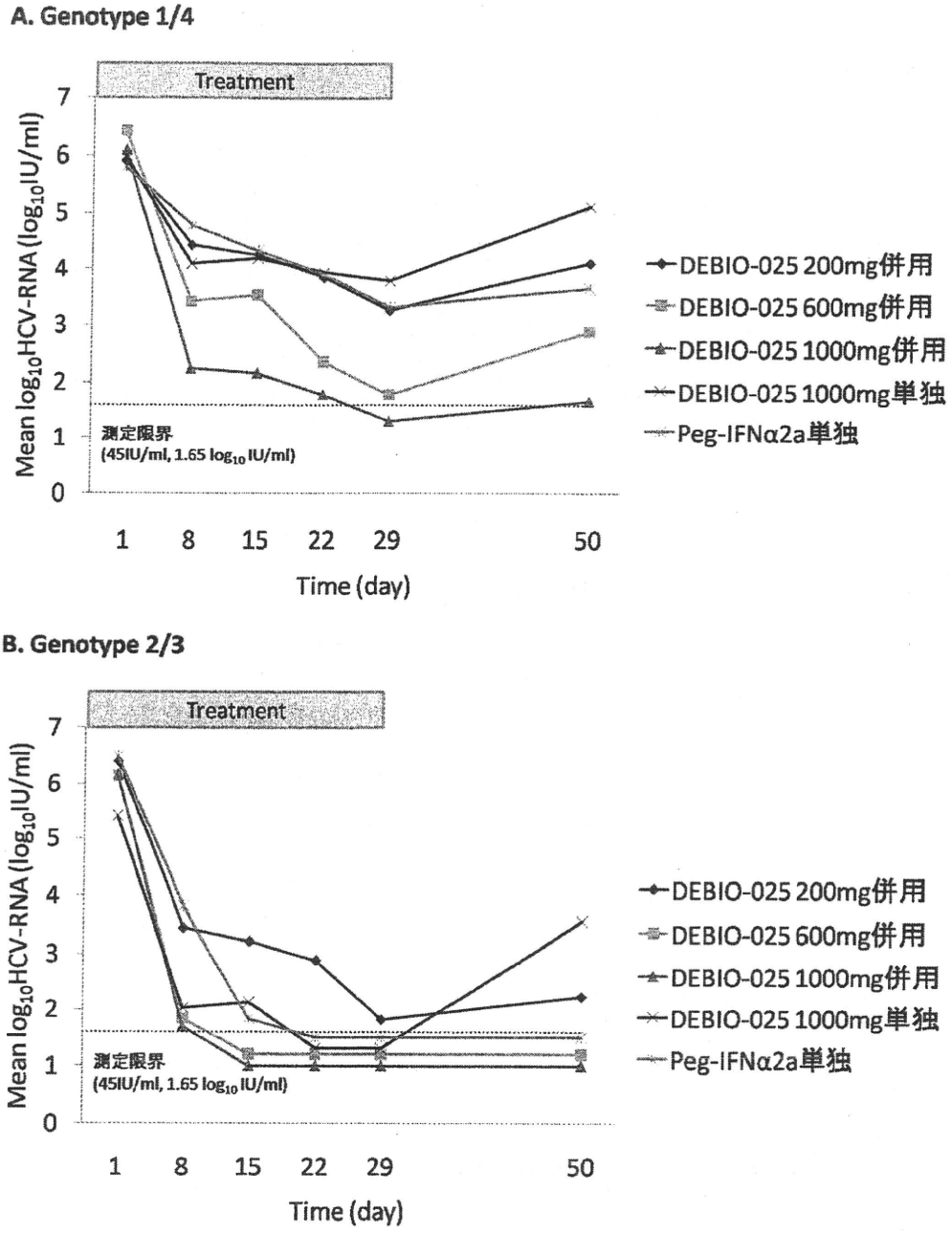

図4　DEBIO-025/Peg-IFNα-2a併用療法の治療効果（文献25より改変）

◆：DEBIO-025 200mg＋Peg-IFNα-2a
■：DEBIO-025 600mg＋Peg-IFNα-2a
▲：DEBIO-025 1,000mg＋Peg-IFNα-2a
×：DEBIO-025 1,000mg
＊：Peg-IFNα-2a

て，Peg-IFNα-2aとの併用による相乗効果が報告された[23]．

2. C型慢性肝炎患者に対する臨床試験

DEBIO-025の単剤投与は，HCV/HIV重感染者（DEBIO-025群：16例，placebo群：3例）に対して行われた[24]．この結果，DEBIO-025（1,200 mg）14日間投与により，平均3.64 $\log_{10}$ copies/ml，最大4.37 $\log_{10}$ copies/mlのHCV-RNA減少が得られ，うち3例でHCV-RNA陰性化を認めたと報告されている．

この結果を受けて，初回投与のC型慢性肝炎に対するDEBIO-025/Peg-IFNα-2a併用療法の第Ⅱ相試験が行われた[25]．対象を，placebo/Peg-IFNα-2a（180 μg/週 ）群とDEBIO-025単剤1,000 mg/日群，Peg-IFNα-2aに併用するDEBIO-025が200 mg/日，600 mg/日，1,000 mg/日の3群，計5群（18例ずつ）に分け，4週投与を行った結果，投与終了時のHCV-RNA減少が，genotype 1/4型で，DEBIO-025 600 mg併用群：4.61±1.88 $\log_{10}$ IU/*ml*，DEBIO-025 1,000 mg併用群：4.75±2.19 $\log_{10}$ IU/*ml*，genotype 2/3型で，DEBIO-025 600 mg併用群：5.91±1.11 $\log_{10}$ IU/*ml*，DEBIO-025 1,000 mg併用群：5.89±0.43 $\log_{10}$ IU/*ml*と，良好な抗ウイルス効果が示された（図4）．また，DEBIO-025 1,000 mg単剤群でもgenotype 1/4型で2.20±2.40 $\log_{10}$ IU/*ml*，genotype 2/3型で4.22±1.33 $\log_{10}$ IU/*ml*と，Peg-IFNα-2a群と同等のHCV-RNA減少が得られている．安全性について，DEBIO-025 200 mg，600 mg併用群ではplacebo群とほぼ同等であり，DEBIO-025 1,000 mg投与群において，36％（13例）に高ビリルビン血症を認めたが，治療終了とともに軽快し，その他重篤な副作用は認めなかった．今後，DEBIO-025/Peg-IFNの第Ⅲ相臨床試験や，Peg-IFN/RBV併用療法において無効であった症例，副作用で脱落した症例などに対する臨床試験など，大規模臨床試験成績の結果が待たれる．

4 おわりに

C型慢性肝炎に対する抗ウイルス療法は，現在のPeg-IFN/RBV併用療法から，HCV選択的酵素阻害剤や免疫修飾剤の登場により，新たな展開を迎えようとしている．宿主因子を標的とした治療は，耐性ウイルスが出現しにくく，またIFNとは異なる機序でのHCVに対する抗ウイルス活性を有している．将来的には，Peg-IFNやRBVとの併用だけではなく，新規経口抗ウイルス薬との併用によるIFN freeのカクテル療法，肝発癌抑止療法などさまざまな臨床応用の可能性も期待され，今後の臨床応用についての報告が待たれる．

文　献

1) Ghany MG, Strader DB, Thomas DL et al : Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 49 : 1335–1374, 2009
2) Hayashi N, Takehara T : Antiviral therapy for chronic hepatitis C: past, present, and future. J Gastroenterol 41 (1) : 17–27, 2006
3) 林　紀夫，小瀬嗣子，平松直樹：C型慢性肝炎治療の変遷．日消誌105 (2) : 175–185, 2008
4) Sarrazin C, Kieffer TL, Bartels D et al : Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology 132 (5) : 1767–1777, 2007
5) McHutchison JG, Everson GT, Gordon SC et al : Telaprevir with peginterferon and RBV for chronic HCV genotype 1 infection. N Engl J Med 360 (18) : 1827–1838, 2009
6) Hézode C, Forestier N, Dusheiko G et al : Telaprevir and peginterferon with or without RBV for chronic HCV infection. N Engl J Med 360 (18) : 1839–1850, 2009
7) McHutchison JG, Manns MP, Muir AJ et al : Telaprevir for previously treated chronic HCV infection. N Engl J Med 362 (14) : 1292–1303, 2010
8) 小瀬嗣子，平松直樹，林　紀夫：高齢者C型肝炎に対するPeg-IFN/Ribavirin併用療法．消化器内科49 (3) : 281–285, 2009
9) Korba BE, Elazar M, Lui P et al : Potential for hepatitis C virus resistance to nitazoxanide or tizoxanide. Antimicrob Agents Chemother 52 : 4069–4071, 2008
10) Korba BE, Montero AB, Farrar K et al : Nitazoxanide, tizoxanide and other thiazolides are potent inhibitors of hepatitis B virus and hepatitis C virus replication. Antiviral Res 77 (1)

: 56–63, 2008
11) Elazar M, Liu M, McKenna SA et al : The anti-hepatitis C agent nitazoxanide induces phosphorylation of eukaryotic initiation factor 2alpha via protein kinase activated by double-stranded RNA activation. Gastroenterology 137 : 1827–1835, 2009
12) Rossignol JF, Kabil SM, El-Gohary Y et al : Clinical trial: randomized,double-blind, placebo-controlled study of nitazoxanide monotherapy for the treatment of patients with chronic hepatitis C genotype 4. Aliment Pharmacol Ther 28 : 574–580, 2008
13) Rossignol JF, Elfert A, El-Gohary Y et al : Improved virologic response in chronic hepatitis C genotype 4 patients given nitazoxanide, peginterferon, and ribavirin. Gastroenterology 136 : 856–862, 2009
14) Rossignol JF, Elfert A, Keeffe EB : Evaluation of a 4 week lead-in phase with nitazoxanide (NTZ) prior to peginterferon (PegIFN) plus NTZ for treatment of chronic hepatitis C: Final report. Hepatology 48 : 344A–345A, 2008
15) Bacon B, Shiffman M, Lim J et al : Phase 2 randomized, double-blind, placebo-controlled study of nitazoxanide plus peginterferon and ribavirin in HCV genotype 1 naïve patients: Week 12 sustained virologic response rate. J Hepatol : 52 (Supple 1) : S463, 2010
16) Shiffman M, Ahmed A, Jacobson I et al : Phase 2 randomized, double-blind, placebo-controlled study of nitazoxanide with peginterferon alfa-2a and ribavirin in nonresponders (NR) with chronic hepatitis C genotype 1: Final report. J Hepatol 52 (Supple 1): S461, 2010
17) Inoue K, Sekiyama K, Yamada M et al : Combined interferon alpha2b and cyclosporin A in the treatment of chronic hepatitis C: controlled trial. J Gastroenterol 38 : 567–572, 2003
18) Watashi K, Hijikata M, Hosaka M et al : Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. Hepatology 38 : 1282–1288, 2003
19) Nakagawa M, Sakamoto N, Enomoto N et al : Specific inhibition of hepatitis C virus replication by cyclosporin A. Biochem Biophys Res Commun 313 : 42–47, 2004
20) Watashi K, Ishii N, Hijikata M et al : Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. Mol Cell 19 : 111–122, 2005
21) Nakagawa M, Sakamoto N, Tanabe Y et al : Suppression of hepatitis C virus replication by cyclosporin a is mediated by blockade of cyclophilins. Gastroenterology 129 : 1031–1041, 2005
22) Paeshuyse J, Kaul A, De Clercq E et al : The non-immunosuppressive cyclosporin DEBIO-025 is a potent inhibitor of hepatitis C virus replication in vitro. Hepatology 43 : 761–770, 2006
23) Inoue K, Umehara T, Ruegg UT et al : Evaluation of a cyclophilin inhibitor in hepatitis C virus-infected chimeric mice in vivo. Hepatology 45 : 921–928, 2007
24) Flisiak R, Horban A, Gallay P et al : The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus. Hepatology 47 : 817–826, 2008
25) Flisiak R, Feinman SV, Jablkowski M et al : The cyclophilin inhibitor Debio 025 combined with PEG IFNalpha2a significantly reduces viral load in treatment-naïve hepatitis C patients. Hepatology 49 : 1460–1468, 2009

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