References

[1] Houghton M, Weiner A, Han J, Kuo G, Choo QL. Molecular biology of the hepatitis C viruses: implications for diagnosis, development and control of viral disease. Hepatology 1991;14:381–388.

[2] Diepolder HM, Zachoval R, Hoffmann RM, Wierenga EA, Santantonio T, Jung MC, et al. Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. Lancet 1995;346:1006–1007.

[3] Cramp ME, Carucci P, Rossol S, Chokshi S, Maertens G, Williams R, et al. Hepatitis C virus (HCV) specific immune responses in anti-HCV positive patients without hepatitis C viraemia. Gut 1999;44:424–429.

[4] Godfrey DI, Hammond KJ, Poulton LD, Smyth MJ, Baxter AG. NKT cells: facts, functions and fallacies. Immunol Today 2000;21:573–583.

[5] Exley MA, Koziel MJ. To be or not to be NKT: natural killer T cells in the liver. Hepatology 2004;40:1033–1040.

[6] Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Motoki K, et al. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. Science 1997;278:1626–1629.

[7] Spada FM, Koezuka Y, Porcelli SA. CD1d-restricted recognition of synthetic glycolipid antigens by human natural killer T cells. J Exp Med 1998;188:1529–1534.

[8] Takahashi T, Chiba S, Nieda M, Azuma T, Ishihara S, Shibata Y, et al. Cutting edge: analysis of human V alpha 24 + CD8 + NK T cells activated by alpha-galactosylceramide-pulsed monocyte-derived dendritic cells. J Immunol 2002;168:3140–3144.

[9] Wilson SB, Kent SC, Patton KT, Orban T, Jackson RA, Exley M, et al. Extreme Th1 bias of invariant Valpha24JalphaQ T cells in type 1 diabetes. Nature 1998;391:177–181.

[10] Araki M, Kondo T, Gumperz JE, Brenner MB, Miyake S, Yamamura T. Th2 bias of CD4 + NKT cells derived from multiple sclerosis in remission. Int Immunol 2003;15:279–288.

[11] Sandberg JK, Fast NM, Palacios EH, Fennelly G, Dobroszycki J, Palumbo P, et al. Selective loss of innate CD4(+) V alpha 24 natural killer T cells in human immunodeficiency virus infection. J Virol 2002;76:7528–7534.

[12] Tahir SM, Cheng O, Shaulov A, Koezuka Y, Bubley GJ, Wilson SB, et al. Loss of IFN-gamma production by invariant NK T cells in advanced cancer. J Immunol 2001;167:4046–4050.

[13] Motsinger A, Haas DW, Stanic AK, Van Kaer L, Joyce S, Unutmaz D. CD1d-restricted human natural killer T cells are highly susceptible to human immunodeficiency virus 1 infection. J Exp Med 2002;195:869–879.

[14] Lucas M, Gadola S, Meier U, Young NT, Harcourt G, Karadimitris A, et al. Frequency and phenotype of circulating Valpha24/Vbeta11 double-positive natural killer T cells during hepatitis C virus infection. J Virol 2003;77:2251–2257.

[15] Karadimitris A, Gadola S, Altamirano M, Brown D, Woolfson A, Klenerman P, et al. Human CD1d-glycolipid tetramers generated by in vitro oxidative refolding chromatography. Proc Natl Acad Sci USA 2001;98:3294–3298.

[16] van der Vliet HJ, Molling JW, von Blomberg BM, Kolgen W, Stam AG, de Gruijl TD, et al. Circulating Valpha24(+)Vbeta11(+) NKT cell numbers and dendritic cell CD1d expression in hepatitis C virus infected patients. Clin Immunol 2005;114:183–189.

[17] Fujii S, Shimizu K, Steinman RM, Dhodapkar MV. Detection and activation of human Valpha24 + natural killer T cells using alpha-galactosyl ceramide-pulsed dendritic cells. J Immunol Methods 2003;272:147–159.

[18] Gumperz JE, Miyake S, Yamamura T, Brenner MB. Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. J Exp Med 2002;195:625–636.

[19] Sandberg JK, Bhardwaj N, Nixon DF. Dominant effector memory characteristics, capacity for dynamic adaptive expansion, and sex bias in the innate Valpha24 NKT cell compartment. Eur J Immunol 2003;33:588–596.

[20] DelaRosa O, Tarazona R, Casado JG, Alonso C, Ostos B, Pena J, et al. Valpha24 + NKT cells are decreased in elderly humans. Exp Gerontol 2002;37:213–217.

[21] de Lalla C, Galli G, Aldrighetti L, Romeo R, Mariani M, Monno A, et al. Production of profibrotic cytokines by invariant NKT cells characterizes cirrhosis progression in chronic viral hepatitis. J Immunol 2004;173:1417–1425.

[22] Kanto T, Hayashi N, Takehara T, Tatsumi T, Kuzushita N, Ito A, et al. Impaired allostimulatory capacity of peripheral blood dendritic cells recovered from hepatitis C virus-infected individuals. J Immunol 1999;162:5584–5591.

[23] Kanto T, Inoue M, Miyatake H, Sato A, Sakakibara M, Yakushijin T, et al. Reduced numbers and impaired ability of myeloid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C virus infection. J Infect Dis 2004;190:1919–1926.

[24] Wynn TA. IL-13 effector functions. Annu Rev Immunol 2003;21:425–456.

[25] Kodera T, McGaha TL, Phelps R, Paul WE, Bona CA. Disrupting the IL-4 gene rescues mice homozygous for the tight-skin mutation from embryonic death and diminishes TGF-beta production by fibroblasts. Proc Natl Acad Sci USA 2002;99:3800–3805.

[26] Bertoletti A, D'Elios MM, Boni C, De Carli M, Zignego AL, Durazzo M, et al. Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. Gastroenterology 1997;112:193–199.

[27] Shields PL, Morland CM, Salmon M, Qin S, Hubscher SG, Adams DH. Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C-infected liver. J Immunol 1999;163:6236–6243.

[28] Harvey CE, Post JJ, Palladinetti P, Freeman AJ, Ffrench RA, Kumar RK, et al. Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates with histological severity and lobular inflammation. J Leukoc Biol 2003;74:360–369.

[29] Apolinario A, Majano PL, Alvarez-Perez E, Saez A, Lozano C, Vargas J, et al. Increased expression of T cell chemokines and their receptors in chronic hepatitis C: relationship with the histological activity of liver disease. Am J Gastroenterol 2002;97:2861–2870.

[30] Durante-Mangoni E, Wang R, Shaulov A, He Q, Nasser I, Afdhal N, et al. Hepatic CD1d expression in hepatitis C virus infection and recognition by resident proinflammatory CD1d-reactive T cells. J Immunol 2004;173:2159–2166.

[31] Kawarabayashi N, Seki S, Hatsuse K, Ohkawa T, Koike Y, Aihara T, et al. Decrease of CD56(+)T cells and natural killer cells in cirrhotic livers with hepatitis C may be involved in their susceptibility to hepatocellular carcinoma. Hepatology 2000;32:962–969.

[32] Nuti S, Rosa D, Valiante NM, Saletti G, Caratozzolo M, Dellabona P, et al. Dynamics of intra-hepatic lymphocytes in chronic hepatitis C: enrichment for Valpha24 + T cells and rapid elimination of effector cells by apoptosis. Eur J Immunol 1998;28:3448–3455.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Hepatology Research 35 (2006) 185–189
HEPATOLOGY RESEARCH
http://www.elsevier.com/locate/hepres

Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy?

Naoki Hiramatsu [a,*], Tsugiko Oze [a], Natsuko Tsuda [a], Nao Kurashige [a], Keisuke Koga [a], Takashi Toyama [a], Masakazu Yasumaru [a], Tatsuya Kanto [a], Tetsuo Takehara [a], Akinori Kasahara [a], Michio Kato [b], Harumasa Yoshihara [c], Kazuhiro Katayama [d], Taizo Hijioka [e], Hideki Hagiwara [f], Shinji Kubota [g], Masahide Oshita [h], Yoshimichi Haruna [i], Eiji Mita, Kunio Suzuki [j], Kazunobu Ishibashi [k], Norio Hayashi [a]

[a] *Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita City, Osaka 565-0871, Japan*
[b] *National Hospital Organization Osaka National Hospital, Osaka, Japan*
[c] *Osaka Rousai Hospital, Osaka, Japan*
[d] *Osaka Kouseinenkin Hospital, Osaka, Japan*
[e] *National Hospital Organization Osaka Minami Medical Center, Osaka, Japan*
[f] *Higashiosaka City Central Hospital, Osaka, Japan*
[g] *Kansai Rousai Hospital, Hyogo, Japan*
[h] *Osaka Police Hospital, Osaka, Japan*
[i] *Osaka General Medical Center, Osaka, Japan*
[j] *Saiseikai Senri Hospital, Osaka, Japan*
[k] *Kaizuka City Hospital, Kaizuka City, Osaka, Japan*

Received 8 January 2006; received in revised form 16 March 2006; accepted 27 March 2006
Available online 4 May 2006

Abstract

The aim of this study was to investigate the efficacy and safety of combination therapy of interferon and ribavirin for aged patients with chronic hepatitis C.

Methods: This study was conducted at Osaka University Hospital and institutions participating in the Osaka Liver Disease Study Group on 329 patients with chronic hepatitis C receiving interferon and ribavirin combination therapy (group A, under 60 year old, $n=199$; group B, 60–64 year old, $n=64$; group C, over 65 year old (mean age, 67.8 ± 2.2 year old, $n=66$)). Of the 293 patients who were tested for HCV serotype and HCV viral loads, 215 had HCV-RNA with serotype 1 and high viral loads (1H) and the other 78 had HCV-RNA with serotype 2 or low viral loads (non-1H).

Results: In per-protocol analysis, the overall SVR rate of 1H patients was 28% (51/184). Among the 1H patients, the SVR rate was significantly lower in group C (16%) and group B (17%) than in group A (34%) ($p<0.05$). The overall SVR rate of non-1H patients was 85% (57/67). No significant difference was found in the SVR rate among group C (79%), group B (100%), and group A (84%). On the other hand, the discontinuance of both drugs due to side effects was 29% (19/66) in group C, 20% (13/64) in group B, and 11% (21/199) in group A, with the discontinuance rates being higher in the older group ($p=0.002$).

Conclusions: In aged chronic hepatitis C patients, interferon and ribavirin combination therapy can be recommended for the non-1H patients who showed a high SVR rate of approximately 65%, but not for the 1H patients.

Keywords: Chronic hepatitis C; Aged patient; Interferon and ribavirin combination therapy

* Corresponding author. Tel.: +81 6 6879 3621; fax: +81 6 6879 3629.
E-mail address: hiramatsu@gh.med.osaka-u.ac.jp (N. Hiramatsu).

1386-6346/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.hepres.2006.03.008

1. Introduction

Hepatitis C virus (HCV) is estimated to infect up to 170 million people worldwide [1]. Long persistence of HCV infection can lead to progression of liver fibrosis causing liver cirrhosis and ultimately hepatocellular carcinoma (HCC) [2,3]. In Japan, it is estimated that two million people are infected with HCV, and more than 30,000 patients die of HCC every year, with approximately 80% being caused by HCV infection [4]. It has been reported that HCV carriers in Japan tend to be old [5], and liver fibrosis progresses in aged patients. Moreover, the risk of HCC increases with progression of liver fibrosis and older age, with the occurrence of HCV-related HCC reaching a peak at around the age of 65 years old [3]. Past studies have made clear that interferon (IFN) therapy is effective for eliminating HCV, and IFN therapy significantly reduces the progression of liver fibrosis [6,7] and the risk of HCC, especially among virologic or biochemical responders [8–10]. Furthermore, recently, several groups have reported that IFN therapy, specially the SVR group, improved the survival of patients with HCV [11,12], also in aged patients [13].

The combination therapy with IFN and ribavirin has been reported to be effective for eliminating HCV compared with IFN monotherapy [14–16], but additional side effects of ribavirin, such as hemolytic anemia, which is not found in IFN monotherapy have been reported, leading to discontinuance of the treatment [17]. For aged patients, sufficient informed consent should be obtained before the start of stronger antiviral therapy with possible severe side effects, because the function of the organs is generally poor, and the adverse effects of IFN therapy have been observed more frequently in older patients [18].

The question arises of whether aged patients with chronic hepatitis C should be treated with the combination therapy of IFN and ribavirin, while IFN monotherapy has been shown to be effective even in aged patients. In this study, we conducted a multi-center, retrospective study of patients with chronic hepatitis C treated by IFN and ribavirin combination therapy, and examined the efficacy and prevalence of side effects to clarify the adaptation of anti-viral treatment for aged patients.

2. Patients and methods

2.1. Patients

The current study was conducted at Osaka University Hospital and the institutions of the Osaka Liver Disease Study Group. The 329 patients with chronic hepatitis C included in this study were treated with combination IFN-α-2b and ribavirin between January 2001 and April 2004. All patients had HCV RNA detectable in serum by the polymerase chain reaction (PCR) method, had elevated ALT (above the upper limit of the normal) and had been histologically proven to have chronic hepatitis. None of the patients were positive for hepatitis B surface antigen and anti-human immunodeficiency virus antibody or had other forms of liver disease (alcoholic liver disease, hepatotoxic drugs, autoimmune hepatitis). This study protocol was carried out according to the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from each patient.

2.2. Determination of HCV RNA levels

Serum HCV-RNA levels were quantified using branched DNA (bDNA) probe assay (version 2; Chiron, Dai-ichi Kagaku, Tokyo) [19,20] or combined PCR assay (Amplicor-HCV monitor assay) [21]. In this study, a high viral load was designated as the condition of a serum HCV-RNA level of more than 10^6 equivalents/ml by bDNA assay or more than 10^5 copies/ml serum by Amplicor-HCV monitor assay [22].

2.3. Treatment schedule

The 329 patients were treated with 10 MU ($n = 79$) or 6 MU ($n = 243$) or 3 MU ($n = 7$) IFN-α-2b intramuscularly every day for the first 2 weeks and the three times a week for the following 22 weeks in combination with ribavirin at a daily dose of 600 or 800 mg, depending on body weight (<60 or ≥60 kg, respectively). The starting doses of ribavirin were 800 mg per day for 178 patients, 600 mg per day for 148 patients, and 400 mg per day for three patients. The ribavirin dose was decreased or stopped in 91 patients (28%) due to side effects. The ribavirin dose of 200 mg was reduced if the hemoglobin value was below 10 g/dl. The ribavirin was stopped if Hb fell bellow 8.5 g/dl. One hundred and five patients continued only IFN therapy for 24 weeks after the combination therapy, because the combination therapy of IFN-α-2b and ribavirin for 48 weeks was not covered by medical insurance in Japan at that time. Patients with persistently undetectable HCV RNA 6 months after completion of treatment were considered to have achieved a sustained virological response.

2.4. Statistical analysis

Age, histological scores before IFN therapy, serum ALT levels, red blood cell (RBC) count, hemoglobin (Hb), white blood cell (WBC) count and platelet (Plt), and creatinine are expressed as mean ± S.D. Statistical analysis for group comparisons was performed by the χ^2-test. The SVR rate was evaluated using the probability proportional to size analysis (PPS analysis) and the intention-to-treat analysis (ITT analysis). A value of $p < 0.05$ (two-tailed) was considered to indicate significance.

3. Results

3.1. Clinical characteristics before combination therapy

The baseline clinical features of the 329 patients are shown in Table 1. At the start of the treatment, 130 patients were 60

Table 1
Baseline characteristics of patients according to age

	Group A ($n=199$)	Group B ($n=64$)	Group C ($n=66$)	p-value
Age (years old)	49.0 ± 8.7	62.0 ± 1.4	67.8 ± 2.2	
Sex (M/F)	142/54[a]	36/28	43/23	[a]$p<0.05$
HCV serotype (1/2/unknown)	142/51/6	53/10/1	54/12/0	N.S.
HCV-RNA (H/L/unknown)	173/12/14	58/2/4	60/5/1	N.S.
1H/non 1H/unknown	125/53/21	45/8/11	45/17/4	
Fibrosis (F 1/F2/F3/F4/unknown)	75/46/33/6/39	26/15/10/2/11	19/15/17/4/11	N.S.
ALT (IU/L)	112 ± 85[b]	91 ± 49	90 ± 57	$p<0.05$[b]
WBC	5330 ± 1570[b]	4970 ± 1390	4760 ± 1120	$p<0.05$[b]
RBC ($\times 10^4$ μl)	458 ± 47[b]	433 ± 45	431 ± 47	$p<0.01$[b]
Hb (g/dl)	14.6 ± 1.5[b]	14.0 ± 1.2	13.7 ± 1.4	$p<0.01$[b]
Plt ($\times 10^4$ μl)	16.0 ± 7.0[b]	14.9 ± 5.3	14.2 ± 4.9	$p<0.05$[b]

Note: Data are given as the mean ± S.D. N.S., not significant. Group A, patients under 60 years of age (gender of three patients were unknown); group B, patients older than 60 years but under 65 years of age; group C, patients older than 65 years of age; 1H group, patients with genotype 1 and high viral load; non-1H group, patients other than 1H group.

[a] Significant level was compared with group B.

[b] Significant levels were compared with group B and group C.

years old or older. One hundred ninety-nine patients were under 60 years old (group A), sixty-four patients were 60–64 years old (group B) and sixty-six patients were 65 years old or older (group C). No significant difference was found in serotype, viral load and histological stage among the three groups. In aged patients, ALT, RBC, Hb, WBC, and Plt were less than in young patients (ALT, $p<0.05$; RBC and Hb, $p<0.01$; WBC and Plt, $p<0.05$). Among the patients, 215 had HCV-RNA with genotype 1 and high viral loads (1H group) and 114 had HCV-RNA with genotype 2 or low viral loads (non-1H group).

3.2. Initial dosage and treatment duration of interferon

Three kinds of IFN dosage were used in this study. Among group A, 10MU, 6MU, and 3MU were administered for 60 patients, 134 patients, and 5 patients; 12, 52, and none among group B, and 8, 56, and 2 among group C. No significant difference was found in the distribution of IFN dosage among each group. The 24 and 48-week treatments (IFN and ribavirin treatment for 24 weeks followed by IFN monotherapy for 24 weeks) were carried out for 102 patients and 75 patients among group A; 37 and 14 among group B; 32 and 16 among group C. The rates of patients receiving the 48-week treatment were similar for the three groups.

3.3. PPS analysis

On PPS analysis, the overall SVR rate of 1H patients was 28% (51/184). The SVR rates were 34% (40/117) for group A, 17% (6/36) for group B, and 16% (5/31) for group C. Among the 1H patients, the SVR rates of group B and C were significantly lower than that for group A ($p<0.05$). The overall SVR rate of non-1H patients was 85% (57/67). No significant difference was found in the SVR rates among group A (84%; 36/43), group B (100%; 5/5), and group C (79%; 11/14) (Fig. 1).

3.4. ITT analysis

On ITT analysis, the SVR rate was 24% (51/215) in 1H patients, being 32% (40/125) for group A, 13% (6/45) for group B, and 11% (5/45) for group C. Among the 1H patients, the SVR rates of group B and C were significantly lower than that for group A (A versus B; $p<0.05$, A versus C; $p<0.01$).

On the other hand, in the non-1H group, the SVR rate was 73% (57/78), being 77% (41/53) for group A, 63% (5/8) for group B, and 65% (11/17) for group C. No significant difference was found among the groups (Fig. 2).

3.5. Adverse effects

The entire treatment schedule without reduction and discontinuance of both drugs was completed by 174 patients (53%). Sixty-two percent (123/199) of the patients in group A, 42% (27/64) in group B, and 36% (24/66) in group C com-

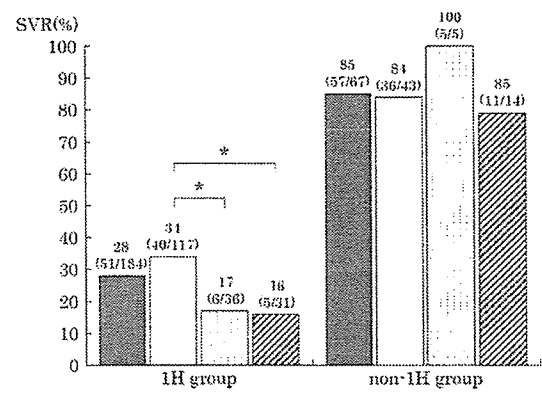

Fig. 1. Efficacy of the combination therapy according to age (PPS analysis). 1H group, patients with genotype 1 and high viral load. Non-1H group, patients not in the 1H group. (■) all patients; (□) group A, patients under 60 years of age; (▒) group B, patients from 60 years and older but under 65 years of age; (▨) group C, patients older than 65 years. Significant levels: *$p<0.05$.

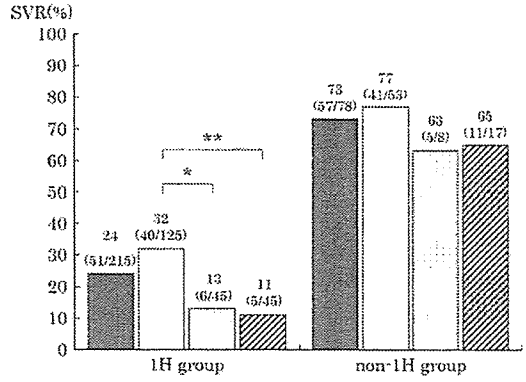

Fig. 2. Efficacy of the combination therapy according to distinction of age (ITT analysis). 1H group, patients with genotype 1 and high viral load. Non-1H group, patients not in the 1H group. (■) all patients; (□) group A, patients under 60 years of age; (▨) group B, patients from 60 years and older but under 65 years of age; (▨) group C, patients older than 65 years. Significant levels: $^{*}p<0.01$; $^{**}p<0.05$.

pleted all treatment schedules (A versus B; $p<0.0001$, A versus C; $p<0.001$). IFN treatment was stopped along with ribavirin in 52 patients (16%), and the IFN dose was decreased in 20 patients (6%). The ribavirin dose was decreased in 72 patients (22%), and stopped without discontinuance of IFN in 20 patients (6%). The discontinuance rate of both drugs was significantly higher in group C (29%, 21/199) and B (20%, 13/64) than group A (11%, 19/66) (Fig. 3).

The reasons for dose reduction and discontinuance of the treatment were anemia, general fatigue, digestive disorder, eczema, neutropenia, and psychological disorder. Among the patients discontinuing both drugs, for those under 60 years old, the major reasons were anemia (32%), general fatigue

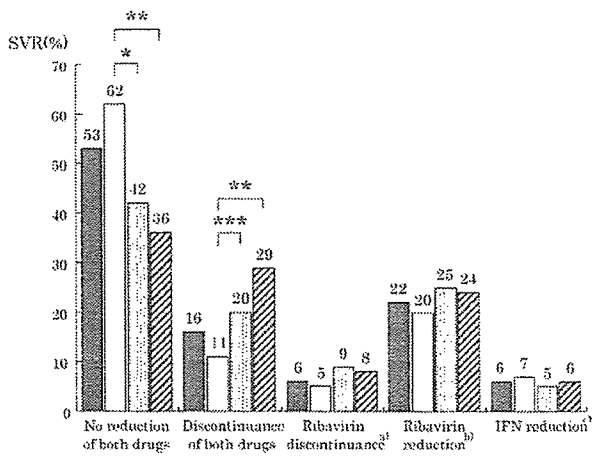

Fig. 3. Dose reduction or discontinuance of IFN and ribavirin. (a) Ribavirin discontinuance without discontinuance of IFN. (b) ribavirin reduction without discontinuance of IFN, and (c) IFN reduction regardless of discontinuance or reduction of ribavirin. (■) all patients; (□) group A, patients under 60 years of age; (▨) group B, patients from 60 years and older but under 65 years of age; (▨) group C, patients older than 65 years. Significant levels: $^{*}p<0.0001$; $^{**}p<0.001$; $^{***}p<0.005$.

(18%), digestive disorder (14%), and psychological disorder (14%). On the other hand, among the patients aged 60 years and older, the discontinuance of therapy due to anemia accounted for approximately 60% (17/28), which was twice as much as those of younger patients, with the difference being significant ($p<0.05$). Other reasons of the discontinuance of therapy among the patients aged 60 years and older were following; digestive disorder (14%), general fatigue (7%), eruption, granulocytopenia, thrombocytopenia, and psychological disorder (4%, respectively). Vascular diseases, such as cerebral bleeding did not appear in this study.

4. Discussion

In Japan, randomized control studies have been performed on the combination therapy of IFN and ribavirin for 24 weeks in patients with chronic hepatitis C, and the combination therapy was approved in 2001. However, the patients in these studies were under 60 years of age. Accordingly, the efficacy and adverse effects of combination therapy for aged patients has been still unclear. Since HCV carriers in Japan are older by 10–20 years than those in the United States and the European countries, it is very important to clarify the actual state of affairs for aged patients with chronic hepatitis C receiving the combination therapy, especially in Japan. These findings should be applicable for patients with chronic hepatitis C in other countries in a few decades, because almost the same efficacy and adverse effects are expected in patients treated by pegylated interferon (peg-IFN) and ribavirin combination therapy. In this study, we examined the efficacy and prevalence of the side effects with the focus on patient age.

The aged patients showed higher rates of discontinuance of IFN and ribavirin and lower rates for no reduction of both drugs than younger patients. The most frequent reason for the discontinuance of both drugs was hemolytic anemia which accounted for 60% of the cases in patients 60 years or older. The progress of anemia was frequently noted in aged patients and resulted in the discontinuation of ribavirin. Hemolytic anemia induced by ribavirin administration has been reported to depend on the plasma ribavirin concentration [23], with a high ribavirin concentration leading to it, and the plasma clearance of ribavirin depending on renal function [24]. A major cause for the advance of anemia in aged patients is due to the fact that renal function is poorer than in younger patients, leading to lower ribavirin clearance. As a result, severe hemolytic anemia can be induced by higher ribavirin concentrations. Therefore, the dosage of ribavirin should be reduced at the beginning of treatment in the aged patients with chronic hepatitis C in order to avoid the discontinuance of ribavirin, because the reduction of ribavirin does not decrease the SVR rate of this therapy.

The SVR difference according to age was observed for 1H patients, but not non-1H patients, when only the patients who completed the treatment were examined (PPS analysis).

That is, the SVR rates were still high for the aged patients of the non-1H group, but lower for the aged patients than the young patients in the 1H group. There are two possible reasons for this. First, the number of patients with no reduction of both drugs was significantly fewer for the patients aged 60–64 years and <60 years than for the patients aged ≥65 years, and the older patients tended to require ribavirin reduction or discontinuance (Fig. 3). Second, the liver fibrosis score tended to be higher in aged patients than in young patients, although the significant difference was not seen in this study (Table 1). These factors can decrease the SVR rates in aged patients in the 1H group, from which it is difficult to eliminate the virus, although the aged patients in the non-1H group whose viruses are easily eliminated were not affected. The results on ITT analysis account for the conclusion of the indication for IFN and ribavirin combination therapy of 24 weeks for aged patients; the patients of the 1H group do not have good application whose SVR is approximately 10%. On the other hand, patients of the non-1H group should be given the combination therapy because of the higher SVR rates of about 65%.

Better efficacy of treatments using new drugs, such as peg-IFN and ribavirin combination therapy or NS3/4 protease inhibitor, is greatly anticipated.

Acknowledgments

Other institutions and participants in the Osaka Liver Disease Study Group (Digestive Disease Study Group of Osaka Renaissance) are: National Hospital Organization Osaka National Hospital, Y. Izumi; Osaka Rousai Hospital, H. Aketa and K. Noda; Osaka Kouseinenkin Hospital, M. Kurokawa and T. Akasaka; Kansai Rousai Hospital, M. Yamamoto; Osaka General Medical Center, T. Inoue; National Hospital Organization Osaka Minami Medical Center, H. Hikita and M. Shigekawa; Osaka Police Hospital, J. Kondo; Kaizuka City Hospital, O. Nishiyama; and Osaka University Graduate School of Medicine, S. Shinzaki, M. Miyazaki, H. Miyatake, I. Itose, S. Egawa and T. Nishida.

This work was supported by a Grant-in-Aid for Research on Hepatitis and BSE from the Ministry of Health Labour and Welfare of Japan, and Scientific Research from the Ministry of Education, Science, and Culture of Japan.

References

[1] Lauer G, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41–52.
[2] Poynard T, Bedossa P, Opolon P, et al. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997;349:825–32.
[3] Hamada H, Yatsuhashi H, Yano K, et al. Impact of aging on the development of hepatocellular carcinoma in patients with posttransfusion chronic hepatitis C. Cancer 2002;95(2):331–9.
[4] Tanaka H, Tsukuma H. Characteristics of Japanese patients with liver cancer—epidemiological study based on comparison between male and female patients. Hepatology Res 2002;24:S11–20.
[5] Yoshizawa H. Trends of hepatitis virus carriers. Hepatology Res 2002;24:S28–39.
[6] Shiratori Y, Imazeki F, Moriyama M, et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 2000;132:517–24.
[7] Hiramatsu N, Hayashi N, Kasahara A, et al. Improvement of liver fibrosis in chronic hepatitis C patients treated with natural interferon alpha. J Hepatol 1995;22:135–42.
[8] Yoshida H, Shiratori Y, Moriyama M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: National surveillance program of chronic and noncirrhotic patients with chronic hepatitis C in Japan. Ann Intern Med 1999;131:174–81.
[9] 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;27:1394–402.
[10] 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;19:1124–30.
[11] 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(2):148–56.
[12] Yoshida H, Arakawa Y, Sata M, et al. Interferon therapy prolonged life expectancy among chronic hepatits C patients. Gastroenterology 2002;123:4483–91.
[13] 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;39:1069–77.
[14] McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alpha 2b alone or combination with ribavirin as initial treatment for chronic hepatitis C. N Engl J Med 1998;229:1485–92.
[15] Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998;352:1426–32.
[16] Hiramatsu N, Kasahara A, Nakanishi F, et al. The significance of interferon and ribavirin combination therapy followed by interferon monotherapy for patients with chronic hepatitis C in Japan. Hepatol Res 2004;29(3):142–7.
[17] Bodenheimer HC, Lindsay KL, Davis GL, et al. Tolerance and efficacy of oral ribavirin treatment of chronic heoatitis C: A multicenter trial. Hepatology 1997;26:437–77.
[18] Okanoue T, Sakamoto S, Itoh Y, et al. Side effects of high-dose interferon therapy for chronic hepatitis C. J Hepatol 1996;25:283–91.
[19] Yuki N, Hayashi N, Kasahara A, et al. Pretreatment viral load and response to prolonged interferon-α course for chronic hepatitis C. J Hepatol 1995;22:457–63.
[20] Lau YN, Davis G, Kniffen J, et al. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. Lancet 1993;341:1501–4.
[21] Shiratori Y, Kato N, Yokosuka O, et al. Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. Gastroenterology 1997;113:558–66.
[22] Tanaka T, Tsukiyama-Kohara K, Yamaguchi K, et al. Significance of specific antibody assay for genotyping of hepatitis C virus. Hepatology 1994;19:1347–53.
[23] Lindahl K, Schvarcz R, Bruchfeld A, et al. Evidence that plasma concentration rather than dose per kilogram body weight predicts ribavirin-induced anemia. J Viral Hepatitis 2004;11:84–7.
[24] Maeda Y, Kiribayashi Y, Moriya T, et al. Dosage adjustment of ribavirin based on renal function in Japanese patients with chronic hepatitis C. Ther Drug Monit 2004;26:9–15.

J Gastroenterol 2006; 41:17–27
DOI 10.1007/s00535-005-1740-7

Journal of Gastroenterology

Review

Antiviral therapy for chronic hepatitis C: past, present, and future

Norio Hayashi and Tetsuo Takehara

Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita 565-0871, Japan

Antiviral therapy for chronic hepatitis C has dramatically advanced since the discovery of the hepatitis C virus (HCV) in 1989 and the introduction of interferon (IFN) monotherapy in the early 1990s. The current standard therapy uses a combination of pegylated IFN and ribavirin. The duration of therapy and response to therapy are HCV genotype-specific. Genotype 1 patients require 48 weeks of the combination therapy for 50% successful viral elimination, while genotype 2 patients require 24 weeks of therapy for 80% or 90% viral elimination. Early viral kinetics after the initiation of therapy is a useful predictor of the sustained virologic response (SVR), which is formally determined at 24 weeks after completion of the treatment. For example, an early virologic response, which is determined by a 2-log reduction of HCV RNA or viral elimination at 12 weeks after the initiation of therapy, is a strong negative predictor of SVR in genotype 1 patients. In contrast, a rapid virologic response of HCV RNA-negative at 4 weeks after the initiation of therapy identifies genotype 2 "super-responders," who may require a shorter period of therapy. Adherence to therapy is one of the most important factors for successful viral clearance. Hematopoietic growth factors such as epoetin and granulocyte-colony stimulating factor help reduce therapy-mediated cytopenia and improve patient compliance, thereby leading to better viral clearance. New types of anti-HCV agents such as HCV protease and polymerase inhibitors are needed for those patients that do not respond to combination therapy.

Introduction

In 1989, the hepatitis C virus (HCV) was discovered in the United States to be the causative agent of posttransfusion non-A, non-B hepatitis by Chiron Corporation (Emeryville, CA, USA).[1] By this discovery, HCV was revealed to be the cause of many hepatic diseases of previously unknown origin. HCV is closely associated with hepatocellular carcinogenesis and death due to chronic liver disease. Epidemiologically speaking, it is estimated that 1.7 million people in Japan and 170 million people worldwide are infected with HCV.[2] Many cases are asymptomatic and result in overt hepatic disease, manifested as hepatic cirrhosis or cancer, only following 20 to 30 years of persistent infection. Thus, HCV infection is of significant concern in terms of public health.

Spontaneous elimination of HCV occurs in approximately 30% of HCV-infected patients within 6 months after infection. However, after this period of time, viral elimination is very rare, with an annual rate of only about 0.2%. Persistent inflammation associated with HCV causes hepatic fibrosis, and as the stage of fibrosis progresses, the risk of cancer increases; annual rates of hepatocarcinogenesis are 0.5% for patients with modest fibrosis and 8% for those with liver cirrhosis.

HCV-associated, progressive hepatic disease can be directly inhibited by interferon (IFN), which is currently the only drug that can eradicate HCV. This review traces the progress of IFN-based therapy for hepatitis C since its introduction and provides a brief overview of the future of HCV treatment.

Introduction of IFN therapy

IFN therapy for hepatitis C dates from 1986, when Hoofnagle et al.[3] reported the normalization of serum alanine aminotransferase (ALT) levels following administration of recombinant human IFNα to patients with non-A, non-B hepatitis. In other words, IFN was shown to be biochemically effective as an anti-inflammatory agent before the discovery of HCV.[4,5]

Received: November 29, 2005 / Accepted: November 30, 2005
Reprint requests to: N. Hayashi

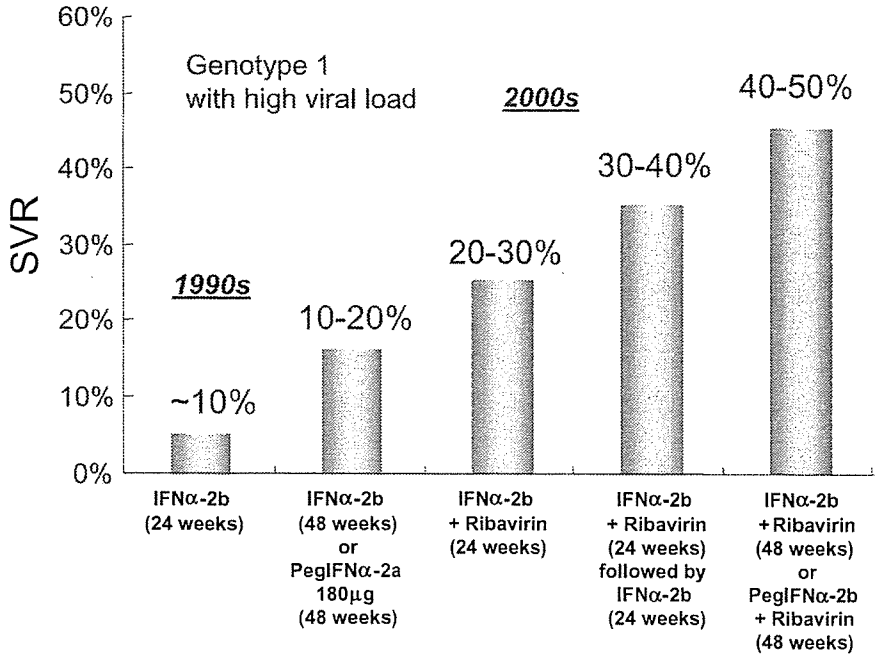

Fig. 1. Milestones of interferon (*IFN*)-based therapy for chronic hepatitis C. Progress in sustained viral clearance for a difficult to treat patient group, with genotype 1 and a high viral load, from the early 1990s. *SVR*, sustained virologic response; *PegIFN*, pegylated interferon

Later, the introduction of a virus identification method using a polymerase chain reaction (PCR) assay revealed cases in which patients became HCV-PCR negative following IFN administration.[6] The normalization of serum ALT levels is associated with viral eradication, with the exception of a few cases. The discovery of these biochemical and virological effects prompted approval of clinical use of IFN against hepatitis C in the United States in 1991 and in Japan in 1992.

The therapeutic responses to IFN can be classified as sustained virologic response (SVR), relapse, or nonresponse. SVR means complete elimination of HCV, which is defined as the loss of detectable HCV RNA during therapy and its continued absence for at least 6 months after the termination of therapy. Relapse is defined as being HCV-negative at the end of IFN treatment but HCV-positive within 6 months after the termination of therapy. Nonresponse is defined as the absence of a HCV-negative condition even with IFN administration. Initial studies showed that after 6 months of 6MU IFN administration to patients with chronic hepatitis C, SVR, relapse, and nonresponse were each observed in one-third of the patients. Subsequent studies revealed that the antiviral effect is determined mainly by viral factors, namely the viral load and the viral genotype.[7,8] Genotypes 1a and 1b are more resistant to IFN therapy than genotypes 2a and 2b, and patients with a high viral load are less likely to respond to IFN than those with a lower viral load. A subgroup analysis of patients treated with IFN monotherapy showed that the SVR rate in genotype 1 patients with a high viral load, accounting for approximately 60% of patients with hepatitis C in Japan, was only 5%. How to improve the therapeutic effect in these patients is the greatest problem for future research and development of IFN therapy (Fig. 1).

Progress of IFN-based therapy

For such resistant cases (patients with genotype 1 and high viral load), extended administration to optimize the total dose of IFN, the introduction of pegylated IFN (PEG-IFN) and coadministration with ribavirin have been used to substantially improve treatment over the past 10 years.

Optimization of the total dose of IFN: extended administration

Two means of increasing the total dose of IFN in resistant cases have been investigated: increasing the dose and extending the administration period. In Japan, patients had usually been given 6MU IFN three times a week for 6 months. Higher doses did not correlate with an increased SVR rate, partly because of the increased incidence of adverse effects and reduced patient compliance. However, extending the administration period proved effective for raising the SVR rate. Kasahara et al.[9] showed that 12 months of administration clearly

increased the SVR rate in genotype 1 patients, compared with 6 months of administration. However, in genotype 2 patients, there was no significant difference between 6 and 12 months of administration. The standard dose of IFN used in Europe and the United States, based on early clinical studies, has been 3 MU three times a week, with the result that European and U.S. therapeutic results after 6 months of IFN monotherapy are generally lower than those in Japan.[10,11] In Europe and the United States, the superiority of the 6-MU dose over a 3-MU dose has been shown by subsequent controlled studies, and many other clinical studies have shown the superiority of 12 months of therapy over 6 months.[12–14] The SVR rate for genotype 1 patients with a high viral load improved with IFN therapy of extended duration, shown first for IFN monotherapy and later for the combination of IFN with ribavirin.[15–17]

Based on these findings, administration of IFN for 12 months was approved early in Europe and the United States. In Japan, the 6-month limit for IFN therapy was removed in 2002, and self-injection of IFN was approved in 2005. These measures make it easier for patients to undergo long-term treatment.

Development of IFN preparations: introduction of PEG-IFN

The type I IFNs include IFNα, IFNβ, IFNω, and IFNλ, all of which share cell-surface receptor and intracellular pathways of action. IFN agents are used in various preparations. In the United States, recombinant IFNα-2b and IFNα-2a were initially approved. In Japan, in addition to these two preparations, natural IFNα and IFNβ can be used. These conventional preparations are considered to be of equal efficacy, although a few differences in the incidences of neutralizing antibodies and adverse effects have been noted.[18] Subsequently, a special agent, consensus IFN, was developed and put into clinical use.[19] It was designed by selecting the most frequently occurring amino acid at each site of the amino acid sequences of 13 known IFNα subtypes. Consensus IFN is considered to have a potent antiviral effect in genotype 1 patients with a high viral load, but it is still considered to be a conventional IFN agent.

Revolutionary progress in the development of IFN agents was recorded with the development of PEG-IFN and its introduction to clinical use. Pegylation is defined as modification of a drug by the addition of an artificial polymer, polyethylene glycol (PEG), for the purpose of delaying drug elimination, lowering its antigenicity, and modifying the drug's effect. Conventional IFN agents, with approximately 8-h elimination half-lives, require a dosing interval of 1 or 2 days to maintain an effective blood concentration.[20,21] The most beneficial effect of PEG-IFN is that it delays drug elimination, making it possible to maintain a stable blood concentration with once-weekly administration.[22] Currently, two PEG-IFN preparations are available: recombinant IFNα-2a and IFNα-2b, which are covalently bound to 40-kDa PEG and 12-kDa PEG, respectively. Both are thought to have about equal efficacy, but they have not been compared in clinical trials.

European and U.S. controlled studies have shown that PEG-IFN agents are generally more effective, both in monotherapy[23–25] and in combination with ribavirin, than conventional IFN agents.[26,27] In Japan, clinical studies have shown that PEG-IFN agents are not inferior to conventional IFN agents. However, no study has shown PEG-IFN agents to be significantly superior with respect to SVR, partly, perhaps, because the usual dose of control IFN agents used in Europe and the United States is 3 MU, which is less than that used in Japan. In sum, PEG-IFN is at least equivalent to conventional IFN in effectiveness, and it appears to be highly tolerable because it can be administered just once a week.

The adverse effects of IFN are classified into two types: those that occur soon after the start of administration, and those that manifest during long-term administration.[28] The former type includes flu-like symptoms, such as a high fever, headache, and myalgia, and abnormal blood test results such as thrombocytopenia and leukopenia. Effects seen with long-term administration include a wide variety of symptoms, such as pruritus, alopecia, fundal hemorrhage, depression, thyroid dysfunction, diabetes mellitus, pulmonary fibrosis, and cardiac arrhythmia. Adverse effects of PEG-IFN are similar to those of conventional IFN and are characterized by mild influenza-like symptoms during the early stage of administration and comparatively severe cytopenia. The occurrence of acute thrombocytopenia in the late stage of administration of PEG-IFNα-2a has also been noted. More caution is needed with respect to the occurrence of adverse effects of PEG-IFN owing to its delayed clearance.

Combination therapy: introduction of ribavirin

Ribavirin, developed in 1972, is a synthetic nucleic acid analog with a purine skeleton. It has antiviral activity in vitro to a wide variety of RNA and DNA viruses, and it is orally administered. Ribavirin has not been approved in Japan as an antiviral agent for monotherapy, but it has been approved in Europe and the United States for various viral diseases, such as severe respiratory syncytial virus infection in children. Its antiviral effect against HCV has not been proved by studies on monotherapy for hepatitis C.[29] In 1998, however, the combination of ribavirin with IFN was reported to have achieved a significantly higher SVR rate compared with IFN

monotherapy.[15,16,30,31] These reports were followed by large-scale clinical studies in Europe and United States[26,27] showing that a combination of PEG-IFN and ribavirin produces better results than one of IFN and ribavirin. With both combinations, 48 weeks of administration to genotype 1 patients achieved a significantly higher SVR rate than 24 weeks of administration.[15,16,32] For other patients, no significant difference was seen between groups receiving 24 or 48 weeks of therapy, and the 24-week administration period was reported to be sufficiently effective.

In Japan, a 48-week, multicenter, randomized, controlled study[33] was conducted on combinations of 6 MU IFNα-2b with ribavirin and 1.5 μg/kg PEG-IFNα-2b with ribavirin administered to genotype 1b patients with a high load of HCV-RNA, determined to be 100 KIU/ml or higher using Amplicor (by the original PCR method). Oral doses of ribavirin administered were 600 mg/day for patients weighing less than 60 kg, 800 mg/day for those weighing at least 60 kg but less than 80 kg, and 1000 mg/day for those weighing 80 kg or more. IFNα-2b was administered six times a week for the first 2 weeks and three times a week for the following 46 weeks, while PEG-IFNα-2b was administered once a week. The results for 506 patients indicated high rates of viral elimination by both therapies. The combination of PEG-IFNα-2b plus ribavirin and that of IFNα-2b and ribavirin achieved SVR in 121/254 patients (47.6%) and 113/252 patients (44.8%), respectively, a difference that was not significant. Based on this phase 3 study, 48 weeks of PEG-IFNα-2b and ribavirin combination therapy was approved for genotype 1 patients with a high viral load in Japan in 2004.

The adverse effects of ribavirin include hemolytic anemia and potential teratogenicity. Caution must be exercised with ribavirin administration when there is coexisting anemia or coronary heart disease. Contraception is also required during administration of ribavirin and up to 6 months after the end of its administration. Ribavirin is contraindicated for patients with renal failure, because it is excreted by the kidney and cannot be eliminated by dialysis.

Recent developments in PEG-IFN and ribavirin therapy

Coadministration of PEG-IFN and ribavirin has been established as the standard regimen of antiviral therapy for hepatitis C,[34] and the following questions next arise. How long should the dosing period be for this combination? How can its adverse effects be ameliorated and the treatment successfully completed? To what extent can this combination treatment be applied? Recent developments offer responses to these questions.

Exploring necessary and sufficient dosing periods: the impact of viral kinetics study

Usually the duration of coadministration of PEG-IFN and ribavirin is 48 weeks for difficult cases (e.g., genotype 1 patients with a high viral load) and 24 weeks for other cases, with expected SVR rates of approximately 50% and 80%, respectively.[32] Some studies have suggested that higher doses of ribavirin based on body weight are more effective for genotype 1, while a lower dose (fixed dose at 800 mg/day) is sufficient for viral genotypes other than genotype 1. To date, a variety of factors, both viral and host, that correlate with a sustained response to the combination therapy have been noted (Fig. 2). In contrast to viral factors, however, most host factors do not have a strong impact on the various treatment regimens. Recently, the viral kinetics after the start of therapy has been noted to be a useful early indicator of viral elimination, which is usually determined 24 weeks after the end of therapy.[20] To find out whether SVR is related to the rate of inhibition of viral replication after the start of PEG-IFN plus ribavirin combination therapy, Davis et al.[35] carried out a retrospective analysis of a controlled clinical study conducted by Manns et al.[26] In the clinical study, PEG-IFNα-2b (1.5 μg/kg per week) and ribavirin (800 mg/day) were coadministered to 511 patients with chronic hepatitis C for 48 weeks. If an early virologic response (EVR) is defined as a viral load decrease of 2 log or more or viral elimination after 12 weeks of treatment, then 71.8% of the patients who experienced EVR—74.4% of all patients—achieved SVR. Importantly, none of the patients who did not experience EVR achieved SVR. Similarly, with therapy with PEG-IFNα-2a (180 μg/week) plus ribavirin (1000 or 1200 mg/day, depending on body weight) for 48 weeks ($n = 453$), only 2 of 63 patients who did not experience EVR achieved SVR.[27] These findings show that EVR has negative predictive value, and therefore, if viral elimination is the aim of the treatment and if adverse effects cannot be negligible, the treatment should be discontinued in patients not displaying EVR. This "12-week rule" applies only to patients with viral genotype 1.[36]

The relationship between the time of becoming HCV-negative and SVR has also been examined in Japan in the above-mentioned clinical study[33] of PEG-IFNα-2b plus ribavirin. SVR rates for patients who became HCV-negative at 4, 12, or 24 weeks (23, 121, and 33 patients, respectively) were 100%, 71.1% and 36.4%, respectively. None of the 15 patients who experienced viral elimination after 24 weeks achieved SVR. Therefore, 24 weeks of additional administration to patients with no viral elimination within the initial 24 weeks produces no benefit.

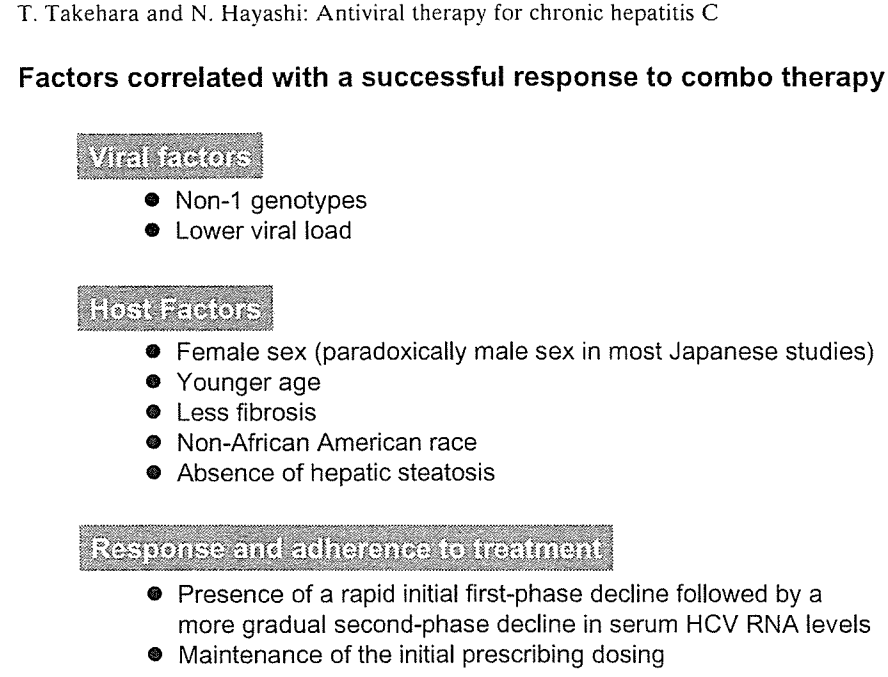

Fig. 2. Factors correlated with a successful response to combination therapy with pegylated interferon and ribavirin in chronic hepatitis C. HCV, hepatitis C virus

Genotype 1 patients who do not experience EVR are very intractable, as shown above. In other words, 48 weeks of therapy with PEG-IFN and ribavirin may be too short to maximize SVR in genotype 1 patients.[37] The usefulness of long-term administration for 48 weeks or longer is being investigated to improve the rate of achievement of SVR in such patients. Buti et al.[38] published a promising report on extending therapy with PEG-IFN plus ribavirin to 72 weeks for late virologic responders. They selected nine genotype 1 patients being treated with PEG-IFNα-2b (1.0μg/kg) plus ribavirin (800mg/day) who cleared HCV RNA between weeks 12 and 24 for therapy prolonged to 72 weeks. Eight patients completed therapy, and at week 24 of follow-up, seven maintained SVR and one had relapsed. A Spanish multicenter, randomized controlled study, in which patients with chronic hepatitis C who did not became HCV negative by 4 weeks of coadministration of PEG-IFNα-2a (180μg/week) and ribavirin (800mg/day) (about two-thirds of all patients) were randomized to groups receiving 48 weeks or 72 weeks of therapy, found that the group receiving 72 weeks of therapy achieved a significantly higher rate of SVR than the group receiving 48 weeks of therapy. On the other hand, a recent clinical trial showed that genotype 1 patients who were HCV RNA-negative after 4 weeks of coadministration of PEG-IFNα-2a (180μg/week) and ribavirin (1000 or 1200mg/day) achieved an SVR rate of 66% with a further 20 weeks of therapy.[39] Unfortunately, this study did not randomize the patients to compare 24 weeks of therapy with a 48-week therapy period. The study, however, does show that 24 weeks of therapy can achieve relatively high rates of viral elimination for these genotype 1 "super-responders."

For other, non-1 viral genotypes, studies are being done to identify a dosing period shorter than 24 weeks that can be used to achieve sufficient SVR. In one study, genotype 2 and 3 patients were given PEG-IFNα-2b (1.0μg/kg each week) and ribavirin (1000 or 1200mg/day, based on body weight), and those who experienced viral elimination after 4 weeks of therapy were assigned to 24-week or 12-week therapy groups. The results showed that the SVR rate for the 12-week group was the same as that for the 24-week group, indicating that 12 weeks of combination therapy is sufficient for these patients.[40] Similar data have also been reported for PEG-IFNα-2a (180μg/week) plus ribavirin (800 to 1200mg/day) therapy.[41]

As mentioned above, for treatment of non-1 viral genotypes and some genotype 1 patients, sufficient SVR rates can be achieved and unnecessary treatment avoided by adopting the dosing period by using the early viral inhibition effect as an indicator. The early viral kinetics can be also applied to identify more difficult to treat patients with viral genotype 1, who can then be given longer treatment to improve SVR rates (Fig. 3).

Reducing cytopenic effects and improving compliance: the use of hematopoietic growth factors

Patient compliance has been noted by many clinical studies to be the largest factor contributing to the therapeutic effect of PEG-IFN plus ribavirin combination

therapy (Fig. 2). Compliance can be divided into those factors related to patient adherence to the regimen and dose interruptions or modifications mandated by the physician in response to cytopenia, rash, gastrointestinal symptoms, or depression. McHutchison et al.[42] outlined an "80:80:80 rule" in genotype 1 patients; that is, the doses of PEG-IFN and ribavirin and the dosing period should exceed 80% of the initial plan to achieve a sufficient SVR rate. Early dose reduction within 12 weeks is more harmful than later dose reduction. To maximize viral clearance of the PEG-IFN and ribavirin combination therapy, countermeasures are needed against adverse effects to improve patient compliance.

Compared with IFN monotherapy, combination therapy is characterized by additional adverse effects represented by hemolytic anemia. If anemia occurs, the dose of ribavirin must be reduced or the administration of ribavirin must be discontinued. To help avoid this adverse effect, attention is being drawn to drug intervention with erythropoietin. An 8-week, double-blind study was conducted in which epoetin alpha 4000 U/week or a placebo was given to patients who experienced a decrease in hemoglobin (Hb) levels to 12 g/dl or less during coadministration of PEG-IFN and ribavirin in the United States, and the dose of ribavirin, Hb levels, and quality of life (QOL) were compared at the end of the study.[43] Compared with the placebo group, the reduction in Hb levels was significantly inhibited in the epoetin alpha group; thus, reduction of the ribavirin dose could be avoided. Inhibition of the reduction in Hb levels also improved QOL.[44] Similarly, granulocyte-colony stimulating factor (G-CSF) is expected to be useful for avoiding leukocytopenia induced by PEG-IFN and ribavirin combination therapy. Prevention of adverse effects with hematopoietic growth factors may be a promising measure to allow the maintenance of the therapy protocol and to improve therapeutic outcomes.

Challenging special patient groups: chronic hepatitis C with persistently normal ALT levels

Persistently normal ALT levels are observed in 20%–30% of chronic HCV-infected patients among the general public. Such patients are sometimes called asymptomatic HCV carriers. Most of them present a picture of histologically minimal or mild chronic hepatitis; it is rare for the liver to be normal. Progression of fibrosis is noted in fewer than 10% of the patients. For this reason, the expression "chronic hepatitis C patients with persistently normal ALT levels" is often preferred to "asymptomatic HCV carriers." There was strong resistance against using IFN therapy for such patients in the 1990s[45,46] for both active and passive reasons. The former included a lower viral elimination effect, or SVR, compared with general hepatitis C patients, and the report of abnormal ALT levels in a high percentage of patients due to IFN therapy in early studies of asymptomatic HCV carriers.[47,48] Recent studies have shown that IFN monotherapy[49] and IFN plus ribavirin combination therapy[50,51] can help patients with persistently normal ALT levels achieve the same level of SVR as patients with abnormal ALT levels. The percentage of patients who display an increase in the ALT level in response to IFN therapy is also lower than that in the early studies.[51] Therefore, the active reasons against using IFN therapy for patients with persistently normal ALT levels can no longer be supported. The passive reason, that there is no evidence of improved long-term prognosis in this patient group by IFN therapy, still remains.

HCV patients with normal ALT levels have been not eligible for large-scale clinical studies, causing there to be a deceptively low level of evidence regarding the efficacy of antiviral therapy in such patients. However, the potential importance of antiviral therapy for such patients has been gaining attention in recent years, and an international, multicenter, randomized, controlled study of PEF-IFNα-2a plus ribavirin combination therapy has been conducted.[52] Eligible participants were 491 HCV RNA-positive patients whose ALT levels measured three times or more at intervals of at least 4 weeks did not exceed the upper limit of the normal ALT range. The patients were randomized at the proportion of 3:3:1 into three groups: patients receiving 24 weeks of therapy with PEF-IFNα-2a (180 μg/week) and ribavirin (800 mg/day), those receiving 48 weeks of PEF-IFNα-2a (180 μg/week) and ribavirin (800 mg/day), and a control group that did not receive any treatment. Acute exacerbation of ALT levels that exceeded ten times the upper limit was observed in two patients (one in the 24-week therapy group and one in the control group). The results regarding treatment effectiveness were identical to those for chronic hepatitis C patients with high ALT levels previously published by Hadziyannis et al.[32] Thus, a dosing period based on the algorithm established for chronic hepatitis C patients with abnormal ALT levels can be recommended for PEG-IFN plus ribavirin combination therapy for HCV-infected patients with persistently normal ALT levels.

Such findings strongly suggest that HCV-infected persons with persistently normal ALT levels should be considered eligible for IFN therapy. The 2004 American Association for the Study of Liver Diseases (AASLD) best-practice guideline[36] recommended as follows: "Regardless of serum aminotransferase levels, the decision to initiate therapy with interferon and ribavirin should be individualized based on the severity of liver diseases by liver biopsy, the potential serious side effects, the likelihood of response, and the presence of comorbid

conditions." What is crucial is not the ALT level but whether to treat the patient if his/her liver disease is not severe.

Future antiviral therapy for hepatitis C

IFN plus ribavirin combination therapy brought about substantial improvement in comparison with the IFN therapy introduced in the 1990s. This combination may lead to high viral elimination primarily because it decreases the incidence of relapse in patients who have become HCV-negative during the therapy. According to an analysis of patient characteristics by the aforementioned Japanese clinical study[33] of PEG-IFNα-2b plus ribavirin combination therapy for genotype 1b patients with high viral load, SVR rates in treatment-naïve patients, relapsers, and nonresponders were 43.1% (59/137 patients) 62.6% (57/91 patients), and 19.2 (5/26 patients), respectively. The fact that relapsers achieved higher SVR rates than treatment-naïve patients suggests that PEG-IFN plus ribavirin combination therapy maximizes the therapeutic effect of IFN and encourages complete viral elimination in IFN-responding patients. On the other hand, the low SVR rate in nonresponders indicates that PEG-IFN plus ribavirin combination therapy is not always useful in patients who do not respond to IFN. To improve SVR rates in such patients, more-effective antiviral agents other than IFN must be developed. Furthermore, as described earlier, PEG-IFN plus ribavirin combination therapy induces a variety of adverse effects. Clearly, safer and better tolerated therapies are needed.

Promising agents for future anti-HCV therapies are classified as HCV-specific inhibitors targeting its protease and polymerase activities, IFN inducers, or less-toxic ribavirin-like agents. A number of drugs are in preclinical or clinical trials.

HCV protease inhibitors

HCV encodes at least four enzymes required for virus replication. They include NS2/3 autoprotease, NS3 helicase, NS3/4A serine protease, and NS5B RNA-dependent RNA polymerase. Intensive work on developing specific inhibitors has focused on the last two.

SCH 503034 is a novel, orally active HCV protease inhibitor that exhibits potent and specific antiviral activity in HCV replicon assays. Recently, a phase 1b clinical trial was conducted for both monotherapy[53] and combination therapy with PEG-IFNα-2b.[54] SCH 503034 exhibited dose-dependent HCV antiviral activity in genotype 1 patients in whom PEG-IFN therapy had previously been unsuccessful. In combination with PEG-IFNα-2b, SCH 503034 had at least an additive effect on HCV suppression. VX-950 is an orally administered highly selective peptidomimetic inhibitor of HCV NS3/4A protease. In a phase 1b clinical trial, VX-950 was well tolerated for 5 to 14 days in both healthy subjects and patients with viral genotype 1, with no serious adverse effects. VX-950 showed a 4.4-log reduction in median HCV RNA at the end of 14 days of therapy.[55]

In addition of its critical role in virus replication, the NS3/4A protease also plays a role in suppressing the cellular antiviral response. Active NS3/4A prevents the phosphorylation and activation of interferon regulatory factor (IRF)-3 and the triggering of downstream IFN-induced antiviral effector genes.[56,57] IRF-3 activity has been shown to be restored by a HCV protease inhibitor. Thus, an effective protease inhibitor may block not only RNA replication but also the ability of HCV to evade innate antiviral responses.

HCV polymerase inhibitors

Valopicitabine (NM283) is a 3′-valyl prodrug of a nucleoside analog that exhibits anti-HCV activity via inhibition of viral RNA polymerase. Valopicitabine is currently in phase 2 clinical development for the treatment of chronic hepatitis C. In a phase 2a trial, valopicitabine demonstrated potent anti-HCV activity when administered in combination with PEG-IFNα-2b, with 4.5-log serum HCV RNA reduction at 6 months and no obvious viral breakthroughs. In a phase 2b clinical trial, the combination therapy was also effective for patients previously unresponsive to PEG-IFN and ribavirin combination therapy.[58]

Since HCV has a higher intrinsic mutation rate than HIV, resistance is expected to be a problem with the use of any type of HCV-specific inhibitor targeting NS3/4A or NS5B proteins. To suppress the risk of a possible escape mutant, combination therapy with PEG-IFN may be better than monotherapy because the former can more efficiently suppress the levels of HCV replication. In the future, a combination of two or three different types of HCV inhibitors may offer a promising approach, similar to HIV cocktail therapy.

Immune modulators

Successful spontaneous clearance of HCV infection is thought to require both innate (e.g., direct antiviral activities by cytokines and natural killer cells) and adaptive (T cell-mediated) immune responses. Chronic HCV infection is characterized by an inadequate immune response that fails to clear the virus.[59] Immune modulators, alone or in combination with direct antiviral agents such as IFN and HCV inhibitors, represent a possible opportunity to improve HCV clearance.

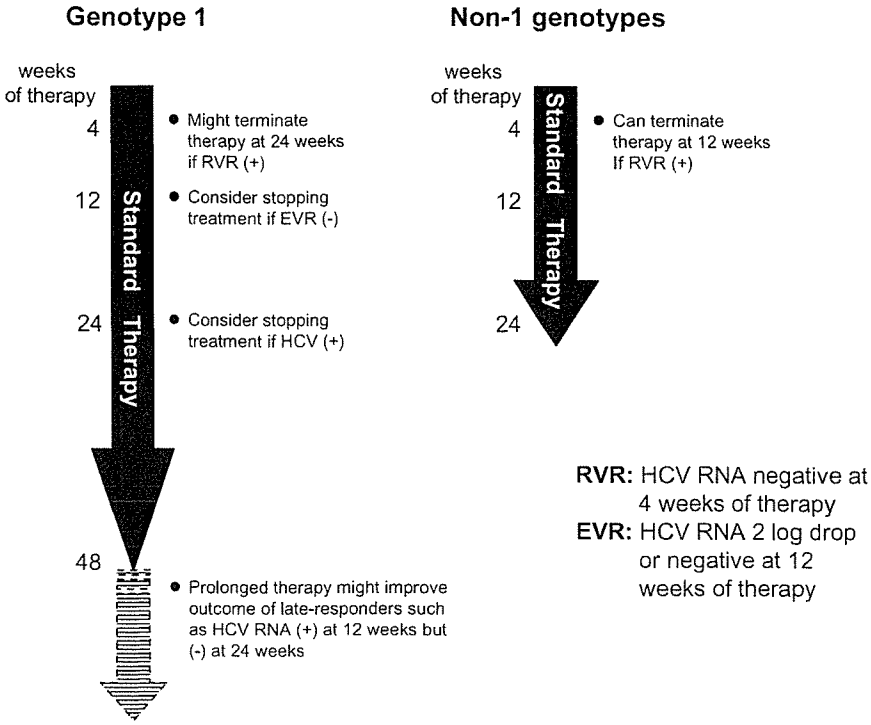

Fig. 3. Various treatment regimens of pegylated IFN and ribavirin combination therapy. *RVR*, rapid virologic response; *EVR*, early virologic response

CPG 10101 is a synthetic agonist of toll-like receptor (TLR) 9. HCV-infected patients receiving CPG 10101 subcutaneously had a more than 1-log reduction in HCV viral load while on therapy.[60] Further development of this agent will continue in conjunction with PEG-IFN and ribavirin.

Isatorbine is a TLR7 agonist. In a proof-of-concept clinical study, intravenous injection of isatorbine once daily for 7 days to patients chronically infected with HCV yielded a significant reduction of serum HCV RNA that correlated with induction of 2′,5′-oligoadenylate synthetase. Recently, the orally available prodrug of isatorbine, ANA975, was developed and studied in healthy phase 1 volunteers and showed promising pharmacokinetics and tolerability.[61]

Ribavirin-like agents

The addition of ribavirin to IFN therapy more than doubled the SVR rate, although its mechanism of action is unknown.[62] Furthermore, higher doses of ribavirin clearly improved response rates in genotype 1 patients.[32,63] However, ribavirin-induced hemolytic anemia is a major obstacle to implementation of a higher dosage regimen and limits its use in patients with comorbidities. To develop a better tolerated combination therapy, ribavirin-like agents lacking a hemolytic effect are needed. Viramidine is a ribavirin prodrug that is metabolized primarily in the liver. In a phase 2 study, fewer patients receiving viramidine developed anemia compared with those given ribavirin, but they also showed lower SVR rates.[64] Phase 3 trials have been undertaken of both PEG-IFNα-2a and PEG-IFNα-2b combined with viramidine in comparison with the combination with ribavirin.

Conclusion: viewpoints other than SVR

IFN treatment of patients with chronic hepatitis C were initially based on observations of its biochemical effects, before the discovery of HCV. Subsequently, evaluation of SVR at 6 months after stopping therapy as a clear end point made it possible to assess therapeutic results in a scientific manner. IFN therapy has been developing over the past decade, with the aim of improving the SVR rate, and higher rates are expected to be achieved with new, more specific antiviral agents.

The question arises as to what the ultimate purpose of hepatitis C treatment is. The answer is that it is the prevention of liver-related death of HCV-infected patients by suppressing progression to decompensated liver disease and liver carcinogenesis (Fig. 4), meaning that hepatitis C is not just an infectious disease, but a potentially serious liver disease. From this point of view, SVR is no more than a surrogate marker—albeit a very strong one—to improve the prognosis of HCV-infected patients. Hepatocellular cancer occurs even in patients

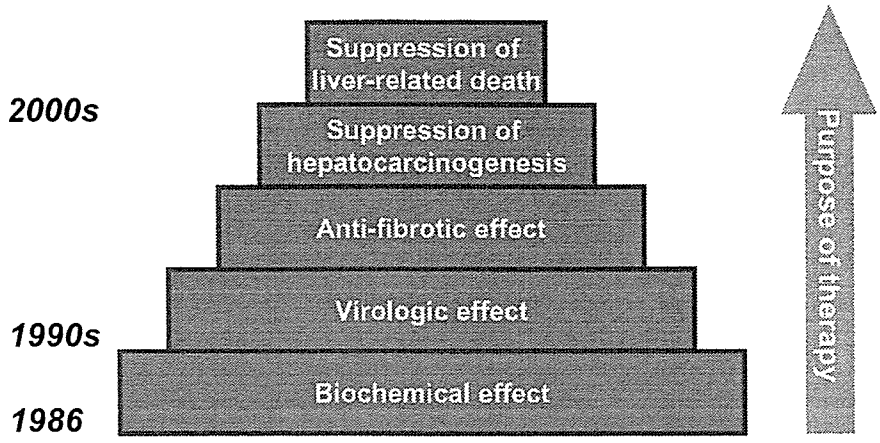

Fig. 4. Effects of IFN-based therapy in relation to the purpose of treatment for chronic hepatitis C. Retrospective analysis of rates of hepatocellular carcinoma and liver-related death after IFN monotherapy have shown a reduction in risk, especially in patients with moderate liver fibrosis[65–69]

who have experienced SVR, although its incidence is substantially lower in those patients than that in untreated patients or nonresponders. Thus, routine hepatocellular cancer screening is essential even after patients have experienced SVR, and early treatment is indispensable if it occurs. On the other hand, the cumulative incidence of hepatocellular carcinoma is clearly suppressed around half in even relapsers at least for 5 years after the termination of therapy compared with that in untreated patients.[65] Therefore, the therapeutic effect of IFN therapy should be evaluated not only on the basis of the SVR rate but also from the more important viewpoint of inhibition of hepatocellular cancer. In this context, repeated IFN therapy, for example every 5 years, for relapsers, and long-term, low-dose IFN therapy for nonresponders should also be considered until a new era dawns of treating hepatitis C with novel anti-HCV agents.

References

1. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244: 359–62.
2. Anonymous. Global surveillance and control of hepatitis C. Report of a WHO consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Viral Hepat 1999;6:35–47.
3. Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Di Bisceglie A, Peters M, et al. Treatment of chronic non-A, non-B hepatitis with recombinant human alpha interferon. A preliminary report. N Engl J Med 1986;315:1575–8.
4. Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC Jr, Perrillo RP, et al. Treatment of chronic hepatitis C with recombinant interferon alpha. A multicenter randomized, controlled trial. N Engl J Med 1989;321:1501–6.
5. Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, et al. Recombinant interferon alpha therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. N Engl J Med 1989;321:1506–10.
6. Hagiwara H, Hayashi N, Mita E, Ueda K, Takehara T, Kasahara A, et al. Detection of hepatitis C virus RNA in serum of patients with chronic hepatitis C treated with interferon-α. Hepatology 1992;15:37–41.
7. Hagiwara H, Hayashi N, Mita E, Takehara T, Kasahara A, Fusamoto H, et al. Quantitative analysis of hepatitis C virus RNA in serum during interferon alpha therapy. Gastroenterology 1993;104:877–883.
8. Mita E, Hayashi N, Hagiwara H, Ueda K, Kanazawa Y, Kasahara A, et al. Predicting interferon therapy efficacy from hepatitis C virus genotype and RNA titer. Dig Dis Sci 1994;39: 977–982.
9. Kasahara A, Hayashi N, Hiramatsu N, Oshita M, Hagiwara H, Katayama K, et al. Ability of prolonged interferon treatment to suppress relapse after cessation of therapy in patients with chronic hepatitis C: a multicenter randomized controlled trial. Hepatology 1995;21:291–7.
10. Marcellin P, Boyer N, Giostra E, Degott C, Courouce AM, Degos F, et al. Recombinant human alpha-interferon in patients with chronic non-A, non-B hepatitis: a multicenter randomized controlled trial from France. Hepatology 1991;13:393–7.
11. Causse X, Godinot H, Chevallier M, Chossegros P, Zoulim F, Ouzan D, et al. Comparison of 1 or 3MU of interferon alpha-2b and placebo in patients with chronic non-A, non-B hepatitis. Gastroenterology 1991;101:497–502.
12. Lindsay KL, Davis GL, Schiff ER, Bodenheimer HC, Balart LA, Dienstag JL, et al. Response to higher dose of interferon alpha-2b in patients with chronic hepatitis C: a randomized multicenter trial. Hepatitis Interventional Therapy Group. Hepatology 1996; 24:1034–40.
13. Poynard T, Leroy V, Cohard M, Thevenot T, Mathurin P, Opolon P, et al. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis: effect of dose and duration. Hepatology 1996;24:778–89.
14. Tassopoulos NC, Karvountzis G, Touloumi G, Delladetsima JK, Papatheodoridis GV, Katsoulidou A, et al. Comparative efficacy of a high or low dose of interferon alpha 2b in chronic hepatitis C: a randomized controlled trial. Am J Gastroenterol 1996;91:1734–8.
15. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomized trial of interferon alpha-2b and ribavirin for 48 weeks or for 24 weeks versus interferon alpha-2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998;352:1426–32.
16. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alpha-2b and ribavirin as initial treatment of relapse of chronic hepatitis C. N Engl J Med 1998;339: 1485–92.

17. Hiramatsu N, Kasahara A, Nakanishi F, Toyama T, Tsujii M, Tsuji S, et al. The significance of interferon and ribavirin combination therapy followed by interferon monotherapy for patients with chronic hepatitis C in Japan. Hepatol Res 2004;29:142–7.
18. Rumi M, Del Ninno E, Parravicini ML, Romeo R, Soffredini R, Donato MF, et al. A prospective randomized trial comparing lymphoblastoid to recombinant interferon alpha 2a as therapy for chronic hepatitis C. Hepatology 1996;24:1366–70.
19. Tong MJ, Reddy KR, Lee WM, Pockros PJ, Hoefs JC, Keeffe EB, et al. Treatment of chronic hepatitis C with consensus interferon: a multicenter, randomized, controlled trial. Consensus Interferon Group. Hepatology 1997;26:747–54.
20. Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-α therapy. Science 1998;282:103–7.
21. Zeuzem S, Schmidt JM, Lee JH, Ruster B, Roth WK. Effect of interferon alpha on the dynamics of hepatitis C virus turnover in vivo. Hepatology 1996;23:366–71.
22. Nieforth KA, Nadeau R, Patel IH, Mould D. Use of an indirect pharmacodynamic stimulation model of MX protein induction to compare in vivo activity of interferon alpha-2a and a polyethylene glycol-modified derivative in healthy subjects. Clin Pharmacol Ther 1996;59:636–46.
23. Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, et al. Peginterferon alpha-2a in patients with chronic hepatitis C. N Engl J Med 2000;343:1666–72.
24. Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, Balart L, et al. Peginterferon alpha-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000;343:1673–80.
25. Lindsay KL, Trepo C, Heintges T, Shiffman ML, Gordon SC, Hoefs JC, et al. Hepatitis Interventional Therapy Group. Lindsay K, et al. A randomized, double-blind trial comparing PEGylated interferon alpha-2b to interferon alpha-2b as initial treatment for chronic hepatitis C. Hepatology 2001;34:395–403.
26. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindoller R, et al. Peginterferon alpha-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet 2001;358:958–65.
27. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–82.
28. Dusheiko G. Side effects of alpha interferon in chronic hepatitis C. Hepatology 1997;26:112S–21S.
29. Bodenheimer HC Jr, Lindsay KL, Davis GL, Lewis JH, Thung SN, Seeff LB. Tolerance and efficacy of oral ribavirin treatment of chronic hepatitis C: a multicenter trial. Hepatology 1997;26:473–7.
30. Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alpha-2b alone or in combination with ribavirin for the treatment or relapse of chronic hepatitis C. N Engl J Med 1998;339:1493–9.
31. Reichard O, Norkrans G, Fryden A, Braconier JH, Sonnerborg A, Weiland O. Randomized double-blind, placebo-controlled trial of interferon alpha-2b with and without ribavirin for chronic hepatitis C. Lancet 1998;351:83–6.
32. Hadziyannis SJ, Sette Jr. H, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-α2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346–55.
33. Iino S, Okita K, Omata M, Kumada H, Hayashi N, Tanikawa K. Clinical efficacy of PEG-Interferon α-2b and ribavirin combination therapy for 48 weeks in chronic hepatitis C patients with genotype 1 and high viral load—retrospective comparison with Interferon α-2b and ribavirin combination therapy for 24 weeks. KanTanSui 2004;49:1099–121.
34. Di Bisceglie AM, Hoofnagle JH. Optimal therapy of hepatitis C. Hepatology 2002;36:S121–7.
35. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alpha-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38:645–52.
36. Strader DB, Wright T, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C. Hepatology 2004;39:1147–71.
37. Drusano GL, Preston SL. A 48-week duration of therapy with pegylated interferon α2b plus ribavirin may be too short to maximize long-term response among patients infected with genotype-1 hepatitis C virus. J Infect Dis 2004;189:964–70.
38. Buti M, Valdes A, Sanchez-Avila F, Esteban R. Extending combination therapy with peginterferon alpha-2a plus ribavirin for genotype 1 chronic hepatitis C late responders: a report of 9 cases. Hepatology 2003;37:1226–7.
39. Ferenci P, Bergholz U, Laferl H, Gurguta C, Maieron A, Gschwantler M, et al. Is shorter treatment with peginterferon alpha-2a (40KD) plus ribavirin possible in HCV genotype 1 "super-responder"? preliminary results of a prospective randomized clinical trial. Hepatology 2005;42:218A.
40. Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, et al. Peginterferon alpha-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med 2005;352:2609–17.
41. Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, et al. Peginterferon-α-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 2005;129:522–7.
42. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002;123:1061–9.
43. Afdhal NH, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, et al. Epoetin alpha maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. Gastroenterology 2004;126:1302–11.
44. Pockros PJ, Shiffman ML, Schiff ER, Sulkowski MS, Younossi Z, Dieterich DT, et al. Epoetin alpha improves quality of life in anemic HCV-infected patients receiving combination therapy. Hepatology 2004;40:1450–8.
45. Marcellin P, Levy S, Erlinger S. Therapy of hepatitis C: patients with normal aminotransferase levels. Hepatology 1997;26:133S–6S.
46. Anonymous. EASL international consensus conference on hepatitis C. Consensus statement. J Hepatol 1999;30:956–62.
47. Serfaty L, Chazouilleres O, Pawlotsky JM, Andreani T, Pellet C, Poupon R. Interferon alpha therapy in patients with chronic hepatitis C and persistently normal aminotransferase activity. Gastroenterology 1996;110:291–5.
48. Sangiovanni A, Morales R, Spinzi G, Rumi M, Casiraghi A, Ceriani R, et al. Interferon alpha treatment of HCV RNA carriers with persistently normal transaminase levels: a pilot randomized controlled study. Hepatology 1998;27:853–6.
49. Rossini A, Ravaggi A, Biasi L, Agostinelli E, Bercich L, Gazzola GB, et al. Virological response to interferon treatment in hepatitis C virus carriers with normal aminotransferase levels and chronic hepatitis. Hepatology 1997;26:1012–7.
50. Di Bisceglie AM, Thompson J, Smith-Wilkaitis N, Brunt EM, Bacon BR. Combination of interferon and ribavirin in chronic hepatitis C: re-treatment of non-responders to interferon. Hepatology 2001;33:704–7.
51. Hui CK, Monto A, Belaye T, Lau E, Wright TL. Outcomes of interferon α and ribavirin treatment for chronic hepatitis C in patients with normal serum aminotransferases. Gut 2003;52:1644–8.
52. Zeuzem S, Diago M, Gane E, Reddy KR, Pockros P, Prati D, et al. Peginterferon alpha-2a (40 kilodaltons) and ribavirin in patients with chronic hepatitis C and normal aminotransferase levels. Gastroenterology 2004;127:1724–32.
53. Zeuzem S, Sarrazin C, Rouzier R, Tarral A, Brion N, Forestier N, et al. Anti-viral activity of SCH 503034, a HCV protease inhibitor, administered as monotherapy in hepatitis C genotype-1 patients

refractory to pegylated interferon (PEG-IFN-α). Hepatology 2005;42 (Suppl. 1):233A.
54. Zeuzem S, Sarrazin C, Wagner F, Rouzier R, Forester N, Gupta S, et al. Combination therapy with the HCV protease inhibitor, SCH 503034, plus Peg-Intron in hepatitis C genotype-1 Peg-Intron non-responder. Hepatology 2005;42 (Suppl. 1):276A.
55. Reesink HW, Zeuzem S, Weegink CJ, Forester N, van Vliet A, McNair LA, et al. Final results of a phase 1b, multiple-dose study of VX-950, a hepatitis C virus protease inhibitor. Hepatology 2005;42 (Suppl. 1):234A.
56. Foy E, Li K, Wang C, Sumpter R Jr, Ikeda M, Lemon SM, et al. Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. Science 2003;300:1145–8.
57. Foy E, Li K, Sumpter R Jr, Loo YM, Johnson CL, Wang C, et al. Control of antiviral defenses through hepatitis C virus disruption of retinoic acid-inducible gene-I signaling. Proc Natl Acad Sci U S A 2005;102:2986–91.
58. O'Brien C, Godofsky E, Rodriguez-Torres M, Afdhal N, Pappas SC, Pockros P, et al. Randomized trial of valopicitabine (NM283), alone or with peginterferon, vs. retreatment with peginterferon plus ribavirin (PEGIFN/RBV) in hepatitis C patients with previous non-response to PEGIFN/RBV: first interim results. Hepatology 2005;42 (Suppl. 1):234A.
59. Takehara T, Hayashi N. Natural killer cells in hepatitis C virus infection: from innate immunity to adaptive immunity. Clin Gastroenterol Hepatol 2005;3:S78–81.
60. Bacon BR, McHutchison JG, Gordon SC, Afdhal NH, Jacobson IM, Shiffman M, et al. Safety, pharmacodynamic (PD) and pharmacokinetic (PK) profiles of CPG 10101 (Actilon), a novel TLR9 agonist: comparison of normal volunteers and HCV infected individuals. Gastroenterology 2005;128 (Suppl 2):P-91.
61. Kerr B, Bauman L, Webber S, Xiang A, Ng J, Kirkovsky L, et al. Pharmacokinetics, safety, and tolerability of the isatorbine oral prodrug ANA975 in a phase 1 healthy volunteer study. Hepatology 2005;42 (Suppl. 1):533A.
62. Feld JJ, Hoofnagle JH. Mechanisms of action of interferon and ribavirin in treatment of hepatitis C. Nature 2005;436:967–72.
63. Lindahl K, Stahle L, Bruchfeld A, Schvarcz R. High-dose ribavirin in combination with standard dose PEGinterferon for treatment of patients with chronic hepatitis C. Hepatology 2005; 41:275–9.
64. Gish RG, Nelson D, Arora S, Fried MW, Reddy KR, Xu Y, et al. Virologic response and safety outcomes in therapy-naïve patients treated for chronic hepatitis C with viramidine in combination with pegylated interferon alpha-2a. Gastroenterology 2005;128 (Suppl 2):P-11.
65. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology 1998;27:1394–402.
66. Kasahara A, Tanaka H, Okanoue T, Imai Y, Tsubouchi H, Yoshioka K, 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;11:148–56.
67. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata 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. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med 1999;131:174–81.
68. Okanoue T, Itoh Y, Minami M, Sakamoto S, Yasui K, Sakamoto M, et al. Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. J Hepatol 1999; 30:653–9.
69. Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, et al. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. Ann Intern Med 1998;129: 94–9.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 346 (2006) 1125–1130
BBRC
www.elsevier.com/locate/ybbrc

Suppressive effect on hepatocyte differentiation of hepatitis C virus core protein

Atsushi Hosui [a], Tetsuo Takehara [a], Kazuyoshi Ohkawa [a,b], Yoshiyuki Kanazawa [a], Tomohide Tatsumi [a], Shinjiro Yamaguchi [a], Ryotaro Sakamori [a], Naoki Hiramatsu [a], Tatsuya Kanto [a,b], Norio Hayashi [a,*]

[a] *Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Suita 565-0871, Japan*
[b] *Department of Dendritic Cell Biology and Clinical Applications, Osaka University Graduate School of Medicine, Suita 565-0871, Japan*

Received 16 May 2006
Available online 26 May 2006

Abstract

The influence of hepatitis C virus (HCV) protein(s) on cellular differentiation remains to be clarified. Using murine normal liver epithelial cells, we investigated whether HCV core protein affects differentiation into hepatocytes. Mock and HCV core-expressing cells were stimulated with oncostatin M (OSM) and dexamethasone, and the degree of differentiation was evaluated by measuring the expression of albumin and tyrosine aminotransferase (TAT). Lower amounts after stimulation were found in HCV core-expressing cells than in mock cells. Phosphorylation of the signal transducer and activator transcription factor 3 (STAT3) was prevented by the HCV core under OSM stimulation. Reporter gene assay revealed that the HCV core/Janus kinase (JAK) interaction directly suppressed the OSM-dependent JAK-STAT signal transduction. Furthermore, expression of OSM receptor β (OSMRβ) after stimulation was prevented by the HCV core. In conclusion, the HCV core may suppress differentiation into hepatocytes via inhibition of the JAK-STAT pathway and OSMRβ expression.

Keywords: Hepatitis C virus; Hepatitis C virus core protein; Hepatic progenitor cells; Differentiation into hepatocytes; Oncostatin M; Signal transducer and activator transcription factor 3 (STAT3); Janus kinase (JAK)-STAT signaling pathway

Hepatitis C virus (HCV) causes persistent infection and leads to chronic hepatitis, liver cirrhosis, and eventually hepatocellular carcinoma (HCC) [1,2]. Chronic liver inflammation induced by HCV brings about repeated hepatocyte apoptosis and liver regeneration, resulting in disease progression. During this process, apoptotic hepatocytes are known to be primarily compensated by replication of intact hepatocytes. However, the replicative activity of hepatocytes has been suggested to be impaired in chronic liver disorder in humans [3] and mice [4]. Proliferation of hepatic progenitor cells (also termed oval cells) and their differentiation into hepatocytes are possible alternative mechanisms for hepatocyte regeneration. Hepatic progenitor cells are a bipotential cellular population that can differentiate into both hepatocytes and bile duct cells. Hepatic progenitor cells have been suggested to play an important role in liver regeneration in many experimental models of the liver injury [5–8]. In addition, it has been reported that hepatic progenitor cells are frequently detected in liver tissues of chronic type C hepatitis patients, and that their numbers increase in parallel with disease severity [9–11]. This suggests that hepatic progenitor cells may be substantially involved in the pathogenesis of chronic type C liver disease.

Among various extracellular factors, dexamethasone, a synthetic glucocorticoid, has been shown to contribute to hepatic maturation of rodent fetal and adult hepatocytes [12,13]. Dexamethasone has been reported to promote

* Corresponding author. Fax: +81 6 6879 3629.
E-mail address: hayashin@gh.med.osaka-u.ac.jp (N. Hayashi).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.05.114

differentiation into hepatocytes of murine hepatic progenitor cells [14]. Oncostatin M (OSM), a member of the interleukin (IL)-6 cytokine family, has been shown to induce development of hepatocytes from fetal hepatic cells in combination with dexamethasone [15]. Recently, OSM has also been reported to inhibit proliferation of rat hepatic progenitor cells, playing a pivotal role in differentiation into hepatocytes [16].

HCV is a plus-stranded RNA virus of approximately 9.5 kb in length [17]. From the HCV genome, at least 10 viral proteins (core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are generated from the precursor protein [18,19]. Recent experimental evidence suggests that the HCV core protein, as well as other HCV proteins, affect various biological functions in the host cell, such as cellular growth, malignant transformation, apoptosis, and signal transduction [20–26]. However, it has not been clarified whether constitutive expression of the HCV core protein affects the differentiation process from hepatic progenitor cells to mature hepatocytes.

To more precisely evaluate this, we used the in vitro culture system of a murine normal liver epithelial cell line stimulated with OSM and dexamethasone to induce differentiation into hepatocytes. We investigated the influence of the HCV core protein on the process of hepatocyte differentiation by comparing the HCV core-expressing cells with negative control (mock) cells.

Materials and methods

Plasmid constructs. Plasmid pCore(1-191)-V5, an HCV core-expressing construct, was prepared from the plasmid pcDNA3.1/V5-HisA (Invitrogen, Co., Ltd.) [25]. Plasmids pCoreMut-V5 and pCoreDel-V5 were generated from pCore(1-191)-V5 by site-directed mutagenesis. These plasmids possessed the mutation (for pCoreMut-V5) or the deletion (for pCoreDel-V5) within a binding site for the Janus kinase (JAK) protein, which had been demonstrated to be located at amino acid positions 79–84 of the HCV core protein [25]. Both pCoreMut-V5 and pCoreDel-V5 encoded mutant types of the HCV core protein that did not allow binding to the JAK protein. Plasmid pAPRELuci contained the three repeats of the acute phase response element (APRE) upstream of the minimal promoter and luciferase gene, which was kindly provided by Dr. T. Hirano (Laboratory of Developmental Immunology, Graduate School of Frontier Biosciences, Osaka University). Plasmid pRLtk (Promega Co.), the seapansy luciferase-expressing plasmid, was used as a transfection efficiency control.

Cell culture and transfection. An embryonic murine liver cell line, BNL CL. 2 (CL2) (No. TIB 73, American Type Culture Collection), has been shown to possess the character of normal liver epithelial cells [27], which are regarded as possible hepatic progenitor cells. The cells were maintained in the Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 μg/ml of streptomycin sulfate, 100 U/ml of penicillin G sodium, and 0.25 μg/ml of amphotericin B in 5% CO_2 at 37 °C. Three independent clones of HCV-core expressing cells (designated CL2 core-I, -II, and -III) and the negative control cells (mock) were established from the CL2 cells, as described elsewhere [25,26]. For induction of hepatic differentiation, 5×10^5 of CL2 mock and core cells were seeded on a 6-cm-diameter culture dish and stimulated with 10 ng/ml of murine OSM (Sigma) and/or 10^{-7} M of dexamethasone (Sigma) every other day. The culture medium was also replaced with the same frequency. In some experiments, the CL2 mock and core cells were treated with 1 μM of Janus kinase (JAK)-specific inhibitor, 2-(1, 1-dimethylethyl)-9-fluoro-3, 6-dihydro-7H-benz[h]-imidaz [4,5-f] isoquinolin-7-one (CN biosciences) [28] every other day 1 h prior to the addition of OSM. Cells were harvested on days 10 or 20 after stimulation for Western blot and the RT-PCR analyses. In the present study, the CL2 core-I cells were mainly used for subsequent experiments. The results were also confirmed with CL2 core-II and -III cells in some experiments (corresponding to Figs. 1 and 2 in this study).

Reporter gene assay. For cotransfection analysis, 8.0×10^4 of the CL2 cells were seeded in a 6-well culture dish and cotransfected with 0.75 μg of the effector plasmid (pCore[1-191]-V5, pCoreMut-V5, pCoreDel-V5 or pcDNA3.1/V5-HisA) with 0.75 μg of the reporter plasmid (pAPRELuci) and 0.1 μg pRLtk. These cells were stimulated with 10 ng/ml of murine OSM or left unstimulated 1 day after transfection. Six hours later, they were lysed and subjected to the dual luciferase assay (Toyo Ink Co., Ltd.). The luciferase activity was normalized for transfection efficiency based on

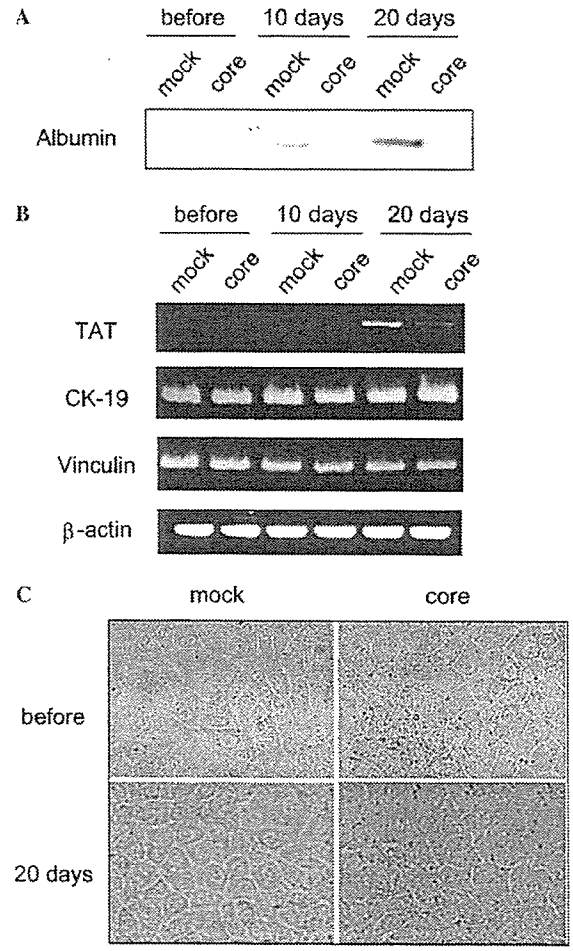

Fig. 1. (A) Detection of albumin in the CL2 mock and core cells under stimulation with OSM and dexamethasone. The cellular protein was harvested before, 10 days after, and 20 days after stimulation and used for Western blot analysis. (B) Detection of TAT, CK-19, and vinculin mRNAs in the CL2 mock and core cells under stimulation with OSM and dexamethasone. The total RNA was extracted before, 10 days after, and 20 days after stimulation and used for the RT-PCR assay. The β-actin mRNA was also measured as a loading control. (C) Microscopic observation of the CL2 mock and core cells under stimulation with OSM and dexamethasone. Phase contrast microscopy of 400 magnifications represents the CL2 mock and core cells before stimulation and 20 days after stimulation with OSM and dexamethasone.

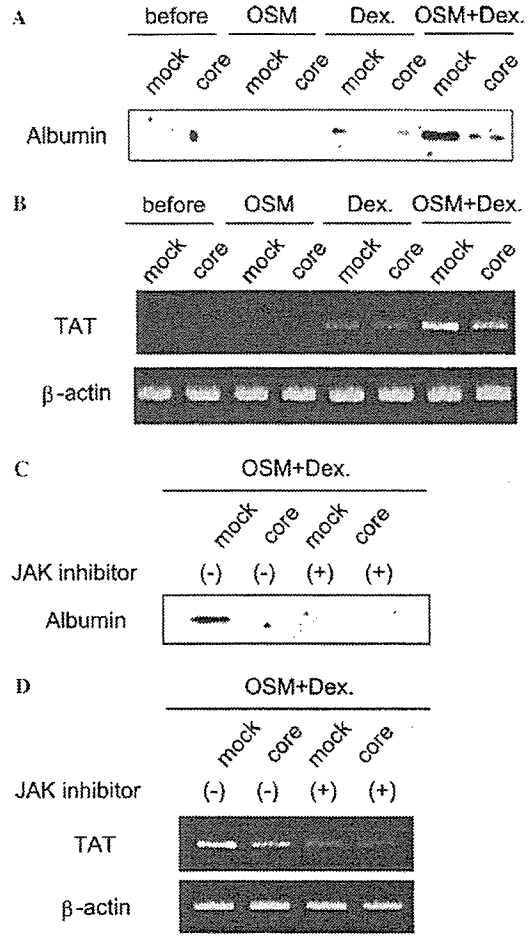

Fig. 2. (A) Detection of albumin in the CL2 mock and core cells under stimulation with OSM alone, dexamethasone alone, or both. The cellular protein was harvested before stimulation and 20 days after stimulation and used for Western blot analysis. (B) Detection of TAT mRNA in the CL2 mock and core cells under stimulation with OSM alone, dexamethasone alone, or both. The total RNA was extracted before stimulation and 20 days after stimulation and used for the RT-PCR assay. The β-actin mRNA was also measured as a loading control. (C) Measurement of albumin expression in the CL2 mock and core cells under stimulation with OSM and dexamethasone in the presence or absence of JAK inhibitor. The cellular protein was harvested 20 days after stimulation and used for Western blot analysis. (D) Measurement of TAT mRNA in the CL2 mock and core cells under stimulation with OSM and dexamethasone in the presence or absence of JAK inhibitor. The total RNA was extracted 20 days after stimulation and used for the RT-PCR assay. The β-actin mRNA was also measured as a loading control.

the result of the seapansy luciferase activity. The relative light unit of the unstimulated sample was regarded as 1, and the sample activities were calculated as multiples of this. All assays were done in triplicate, and the values were expressed as means ± SD.

Western blot analysis. The total cellular protein was extracted with the RIPA buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 50 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 100 μg/ml phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, and 50 mM sodium fluoride in phosphate-buffered saline (pH 7.4) [29]. Twenty micrograms of protein was separated with SDS–polyacrylamide gel electrophoresis and blotted onto polyvinylidene difluoride membrane (Hybond P; Amersham Pharmacia Biotech Co., Ltd.). After blocking with milk, the membrane was incubated with a first antibody, followed by incubation with horseradish peroxidase-labeled immunoglobulin as a second antibody. The immune complex was detected by an enhanced chemiluminescent assay (Super Signal, Pierce). An antibody against signal transducer and activator transcription factor 3 (STAT3) was purchased from Santa Cruz Biotechnology, and an antibody against tyrosine phosphorylated STAT3 (pY^{705}STAT3; pSTAT3) came from Cell Signaling Technology. Antibodies against albumin and OSM receptor β (OSMRβ) were from Upstate Biotechnology.

PCR Analysis. The expression levels of tyrosine aminotransferase (TAT), cytokeratin (CK)-19, and vinculin mRNAs were analyzed by PCR assay. The total cellular RNA was extracted from the CL2 mock and core cells using an Isogen kit (Nippon Gene Co.) based on the guanidine–isothiocyanate method. Reverse transcription (RT) was performed with 1 μg of the RNA sample using the mutated Moloney murine leukemia virus reverse transcriptase (ReverTra Ace, Toyobo) and the oligo$(dT)_{20}$ primer (Toyobo). The cDNA was subsequently amplified with Taq/Pwo DNA polymerase (Expand High Fidelity PLUS PCR System, Roche Diagnostics). The specific primer sets are 5′-GGGGACCCTACTG TGTTTGG-3′ and 5′-GAGGCAGTGGACAGACTGCT-3′ for TAT, 5′-GTCCTACAGATTGACAATGC-3′ and 5′-CACGCTCTGGATCTG TGACAG-3′ for CK-19, and 5′-CGACTAACTGATGAGCTGGC-3′ and 5′-CACAGACTGCATGAGGTTCT-3′ for vinculin. Each cDNA was amplified by 35 PCR cycles involving denaturation at 94 °C for 15 s, annealing at 55 °C for 30 s, and extension for 1 min, followed by final extension at 72 °C for 10 min. As an internal control, the β-actin mRNA was also amplified by 25 PCR cycles. The PCR products were subjected to the agarose gel electrophoresis and visualized by ethidium bromide staining. Under these assay conditions, the mRNA expression levels could be semiquantitated according to the band intensities.

Statistical analysis. Statistical analysis was performed using the non-paired *t* test as appropriate. *P* values less than 0.05 were considered to be statistically significant.

Results and discussion

In the present study, a murine normal liver epithelial cell line, CL2 [27], was stimulated with OSM and dexamethasone to induce differentiation into hepatocytes. To investigate the effect of the HCV core protein on the process of hepatocyte differentiation, the CL2 core cells, which constitutively expressed the HCV core protein [25,26], were compared with the negative control (mock) cells. We first examined the expression levels of hepatocyte-specific marker genes, albumin and TAT, and the bile duct epithelial cell-specific marker genes, CK-19 and vinculin, in the CL2 mock and core cells before and after stimulation with OSM and dexamethasone (Fig. 1A and B). Expression of albumin and TAT was considerably induced after the stimulation in both cells. CK-19 and vinculin were expressed before the stimulation, but their levels were not increased after the stimulation. Thus, the CL2 cells were found to express dual markers of hepatocyte and biliary lineages, which is a known phenotypic feature of hepatic progenitor cells. It was also shown that the stimulation with OSM and dexamethasone could induce differentiation into hepatocytes but not into bile duct cells in the CL2 mock and core cells. According to this, our system using the murine embryonic liver-derived "hepatic progenitor-like" CL2 cells may, to a certain extent, reproduce the process from the hepatic progenitor cells to mature hepatocytes.