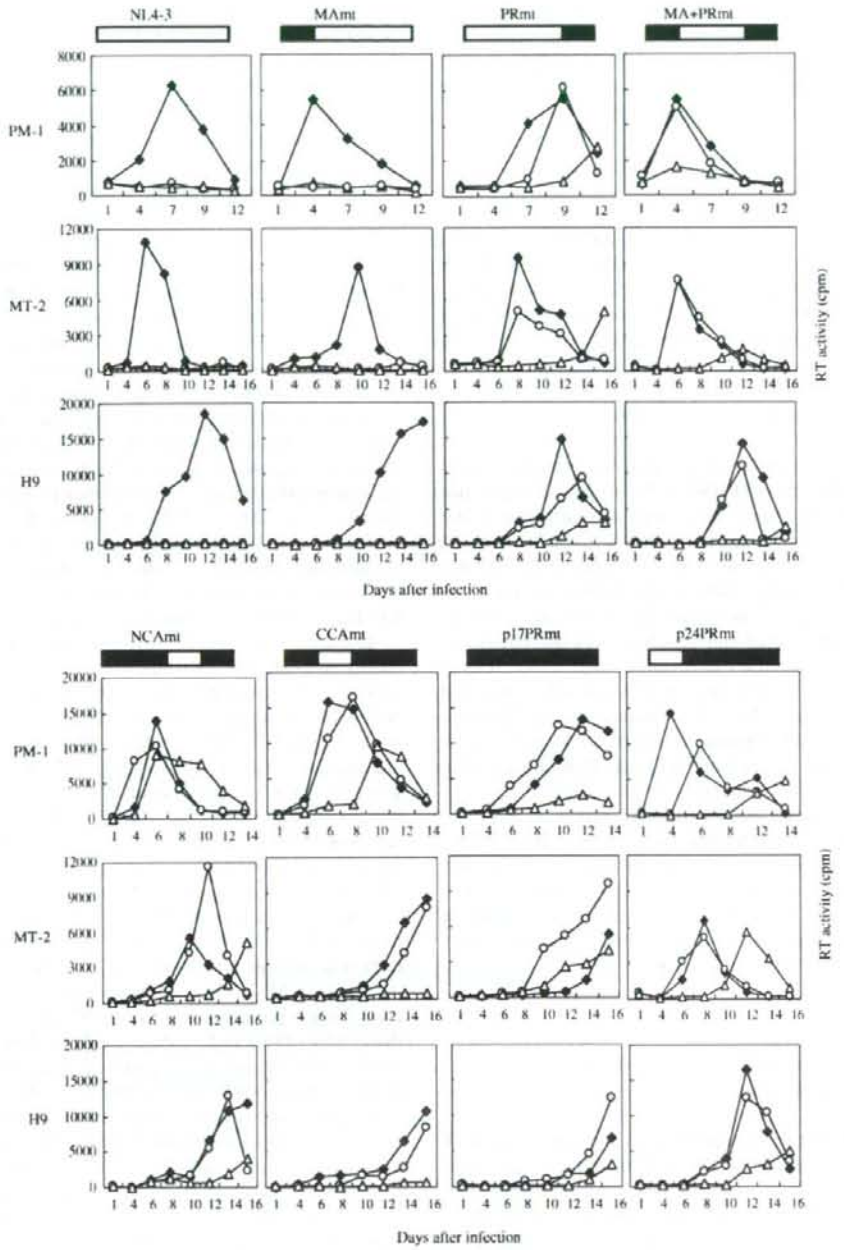

Fig. 3. Effects of NFV on replication capability of recombinant HIV-1s. PM-1, MT-2, and H9 cells (2×10^4 cells) were exposed to 0.2 ml of cell-free supernatant containing each HIV-1 clone (2×10^5 ^{32}P cpm of RT activity), washed once, and cultured in 0.2 ml of medium in the absence (closed diamonds) and presence of NFV (0.1 μM; open circles, 1 μM; open triangles). Half volume of the culture medium was changed every 2 or 3 days, and the supernatant was kept at −80 C until the measurement of RT activity. Each experiment was carried out in duplicate and repeated three times, and representative data are shown.

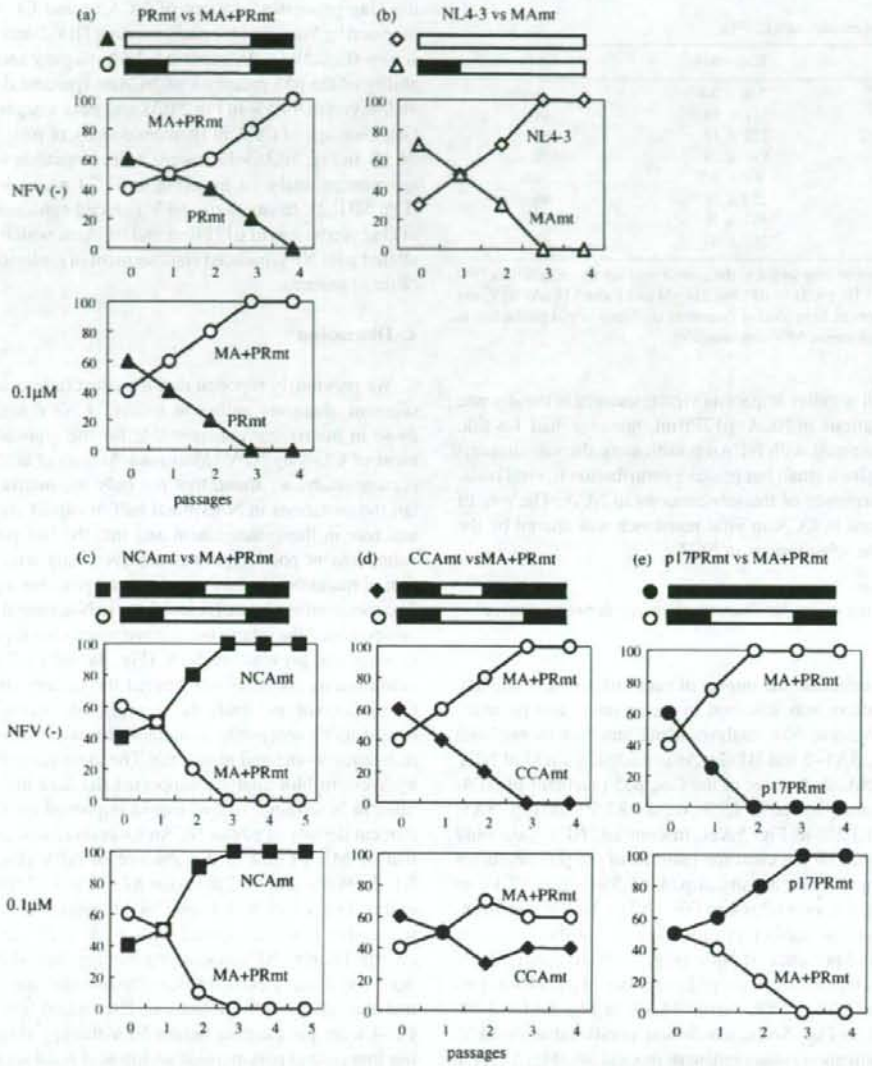

Fig. 4. One-to-one competitive HIV-1 replication assay. H9 cells (2×10^5 cells) were incubated with two recombinant HIV-1s (each of 100 $TCID_{50}$) simultaneously at 37 °C for 16 h, washed with PBS twice, and cultured in the absence and presence of 0.1 μM of NFV. At 7 days post-infection, the culture supernatant was used to infect fresh uninfected H9 cells. The cells harvested at each passage were subjected to direct DNA sequencing, and the viral population changes were determined by the relative peak height in the sequencing electrophoregram. The persistence of the original amino acid substitutions was confirmed for all infectious clones used in this assay. (a) PRmt vs. MA + PRmt; (b) NL4-3 vs. MAmt; (c) NCAmt vs. MA + PRmt; (d) CCAmt vs. MA + PRmt; (e) p17PRmt vs. MA + PRmt.

3.2. Gag substitutions conferring NFV resistance

To analyze the role of the substitutions in the matrix, NCA, and CCA in NFV resistance, IC_{50}s of NFV for the HIV-1 clones described above were determined by using MT-2 cells (Zhang et al., 1997). MAmt (IC_{50}: 9.4 nM) showed 1.9-fold resistance against NFV compared with NL4-3 (5.0 nM), and p17PRmt (893 nM) showed 3.3-fold resistance compared with p24PRmt (273 nM) (Table 1), indicating that the substitutions in the matrix may make a small contribution to the viral resistance against NFV. NCAmt (483 nM) had 2.1-fold resistance against NFV compared with MA + PRmt (229 nM), and p17PRmt had 4.1-fold resistance compared with CCAmt (217 nM), indicating that the substitutions in NCA gave positive impact on viral resistance. Interestingly, CCAmt showed 0.95-fold resistance against NFV compared with MA + PRmt, indicating that substitutions in CCA

Table 1
NFV resistance of recombinant HIV-1s

HIV-1	IC_{50} (nM)	Fold-resistance
NL4-3	5.0 ± 0.4	1.0
PRmt	241 ± 34	48
p24PRmt	273 ± 13	55
p17PRmt	893 ± 28	179
MAmt	9.4 ± 3.3	1.9
MA + PRmt	229 ± 21	46
NCAmt	483 ± 26	97
CCAmt	217 ± 32	43

The concentrations of drug added to the growth medium for calculation of the IC_{50}s were 0, 1, 3.16, 10, 31.6, 100, and 316 nM and 1 and 3.16 μM NFV, and the IC_{50}s were derived from plots of percent of inhibition of p24 production in culture supernatant versus NFV concentration.

may give small negative impact on viral resistance in the absence of the substitutions in NCA. p17PRmt, however, had 1.8-fold resistance compared with NCAmt, indicating the substitutions in CCA may give a small but positive contribution to viral resistance in the presence of the substitutions in NCA. The role of the substitutions in CCA in viral resistance was altered by the presence of the substitutions in NCA.

3.3. Gag substitutions facilitating cleavage between matrix and capsid

To further delineate the impact of each substitution, the Gag processing pattern was assessed in the absence and presence of NFV by Western blot analysis using anti-p24 monoclonal antibody (Fig. 5A1–2 and B1–2). As expected, 0.1 μM of NFV effectively blocked cleavage of the Gag p55 precursor of NL4-3 (percent density of p24; 4.7% versus 87.5% in Fig. 5A1; 4.2% versus 83.3% in Fig. 5A2). In contrast, NFV gave only a small influence on the cleavage patterns of the p55 precursor of MA + PRmt (percent density of p24; 65.5% versus 87.4% in Fig. 5A1; 77.8% versus 92.6% in Fig. 5A2), which is consistent with the indistinguishable replication kinetics of this mutant in the absence and presence of NFV (Fig. 3). Interestingly, NFV enhanced the cleavability of the p55 precursor of p17PRmt (percent density of p24; 94.8% versus 74.3% in Fig. 5A1; 72.2% versus 54.1% in Fig. 5A2), which was paralleled with NFV-dependent replication enhancement of this mutant (Fig. 3). NFV also gave a small positive effect on the cleavability of the p55 precursor of NCAmt (percent density of p24; 97.1% versus 94.6% in Fig. 5B1; 97.5% versus 96.2% in Fig. 5B2), which was paralleled with the partial enhancement of replication with NFV (Fig. 3). Furthermore, percent density of p24 of NCAmt was increased compared with that of MA + PRmt (percent density of p24; 94.6% and 96.2% versus 87.4% and 92.6% in the absence of NFV; 97.1% and 97.5% versus 65.5% and 77.8% in the presence of 0.1 μM NFV), suggesting that the substitutions in NCA play a significant role in Gag cleavability. Finally, NFV decreased percent density of p24 of CCAmt (percent density of p24; 68.9% versus 78.2% in Fig. 5B1; 45.3% versus 79.0% in Fig. 5B2), which was paralleled with NFV-induced delay of replication kinetics (Fig. 3). For further confirmation, the Gag processing pattern of NCAmt and CCAmt was also assessed by Western blot analysis using HIV-1-infected patient's serum (Fig. 5B3). As expected, NFV slightly increased cleavability of the p55 precursor of NCAmt (percent density of p24; 96.9% versus 94.5% in Fig. 5B3), and gave a negative impact on Gag cleavage of CCAmt (percent density of p24; 41.9% versus 74.3% in Fig. 5B3), which were well compatible with the cleavage pattern analyzed by using anti-p24 monoclonal antibody (Fig. 5B1, 2). In summary, NFV induced enhanced cleavability of Gag precursors of p17PRmt and NCAmt, which was well paralleled with NFV-induced enhancement of replication capability of these mutants.

4. Discussion

We previously reported that the substitutions in p6-protease segment alone are sufficient to confer NFV resistance while those in matrix are indispensable for the replication enhancement of CL-4 by NFV (Matsuoka-Aizawa et al., 2003). In the present study, we found that not only the matrix substitutions but the mutations in N-terminal half of capsid also played critical role in the enhancement and that the full potential of the enhancement phenotype was achieved only with the cooperation of mutations in the entire Gag and protease region of CL-4. The substitutions in matrix and those in N-terminal half of capsid compensated the otherwise compromised viral replication in the absence and presence of NFV (Fig. 4a and c). Probably, these substitutions cooperatively altered the tertiary structure of the Gag precursor and made the cleavage site between matrix and capsid more accessible to mutant protease harboring multiple resistance-associated mutations. The cleavage pattern analyzed by Western blot analysis supported the idea that the substitutions in N-terminal half of capsid improved the Gag cleavage. Percent density of p24 of NCAmt was increased compared with that of MA + PRmt in the absence of NFV (Fig. 5A1–2 and B1–2; 94.6% and 96.2% versus 87.4% and 92.6%). It is worth noting that CL-4 had a total of 56 amino acid substitutions in *gag-pro* genes compared with NL4-3; 22 substitutions had emerged during NFV-containing therapy, and 34 other substitutions had already existed before the introduction of the therapy, and that all the substitutions in N-terminal half of capsid of CL-4 were pre-existing before NFV-therapy (Fig. 1), suggesting that certain polymorphic amino acid residues seen in HIV-1 clinical isolates were associated with drug resistance. Interestingly, the amino acid insertion at the same site of the matrix of CL-4 compared with NL4-3 (Fig. 1; amino acids 121–125 in MA, QQAAA) was reported to increase viral replication harboring mutant protease by improving otherwise impaired Gag processing (Tamiya et al., 2004). Gatanaga et al. also reported that a polymorphic substitution in N-terminal half of capsid was indispensable for the development of high multitude of resistance against PIs (Gatanaga et al., 2002), though CL-4 did not harbor the same substitution. It is also known that certain drug-resistance-conferring amino acid substitutions found in one subtype HIV-1 isolated from patients under therapy may be detected in HIV-1 of other subtypes from untreated individuals (Cornelissen et al., 1997; Quinones-Mateu et al., 1998). More-

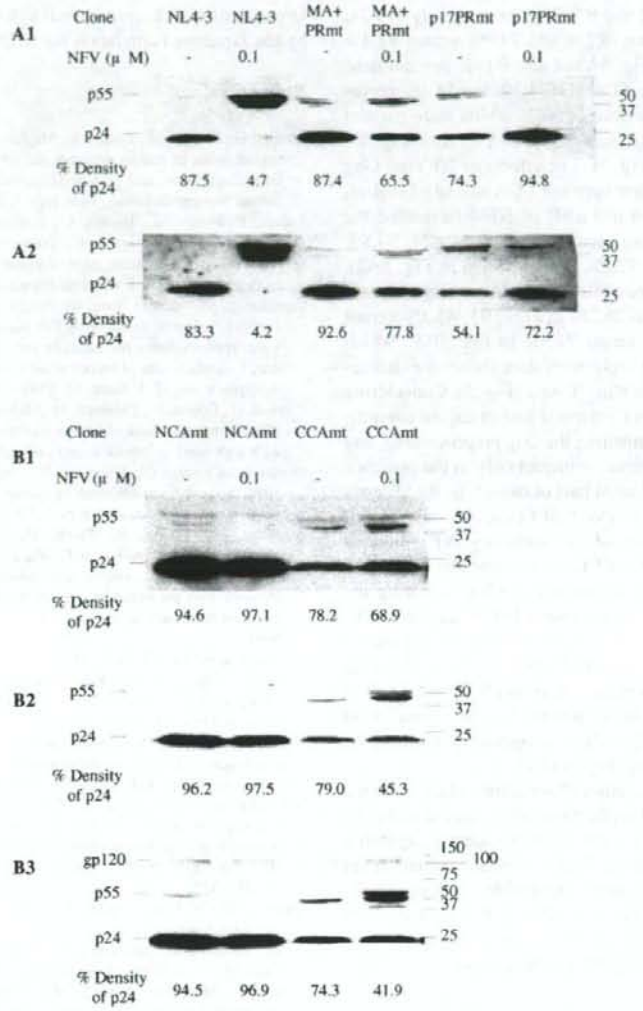

Fig. 5. Western blot analysis in the absence and presence of NFV. HeLa cells were transfected with each of full-length molecular clones and cultured in the absence and presence of 0.1 μM NFV. At 48 h post-transfection, virions in the culture supernatant (6×10^5 cpm of RT activity) were harvested and subjected to Western blot analysis. Gag proteins were visualized by using anti-p24 monoclonal antibody (A1-2, B1-2) and HIV-1-infected patient's serum (B3). Percent density of p24 was calculated as 100 × (p24 signal density/total Gag product signal densities) in a Western blot.

over, a recent study of Colson et al. revealed that HIV-2 strains harbor specific patterns of natural polymorphism and resistance (Colson et al., 2004). HIVs seem to acquire drug-resistance by utilizing the pre-existing polymorphic mutations and by coordinating the development of multiple substitutions.

Furthermore, the substitutions in N-terminal half of capsid of CL-4 altered the effect of NFV on viral replication. Sub-inhibitory concentration (0.1 μM) of NFV slightly accelerated the Gag precursor cleavage of NCAmt (percent density of p24; 97.1% versus 94.6% in Fig. 5B1; 97.5% versus 96.2% in Fig. 5B2; 96.9% versus 94.5% in Fig. 5B3), which was paralleled with the partial replication enhancement with NFV (Fig. 3), though it showed inhibitory effect in Gag processing of MA + PRmt (percent density of p24; 65.5% versus 87.4% in Fig. 5A1; 77.8% versus 92.6% in Fig. 5A2). Therefore, one of the mechanisms of viral replication enhancement with NFV is the improved processing of Gag harboring the substitutions in N-terminal half of capsid of CL-4 cooperated with the substitutions in the matrix. On the other hand, the role of the substitutions in C-terminal half of capsid seemed different, though they were also indispensable for the full potential of replication enhancement with NFV. They impaired the cleavability of Gag precursor

of MA + PRmt (Fig. 5A1–2 and B1–2; percent density of p24; CCAmt versus MA + PRmt = 78.2% and 79.0% versus 87.4% and 92.6%) and NCAmt (Fig. 5A1–2 and B1–2; percent density of p24; p17PRmt versus NCAmt = 74.3% and 54.1% versus 94.6% and 96.2%) in the absence of NFV, which were parallel with viral replication data (CCAmt versus MA + PRmt, Fig. 4d; p17PRmt versus NCAmt, Fig. 3). The effects of NFV on Gag cleavage pattern were different between CCAmt and p17PRmt; sub-inhibitory concentration (0.1 μM) of NFV facilitated the Gag cleavability of p17PRmt (percent density of p24; 94.8% versus 74.3% in Fig. 5A1; 72.2% versus 54.1% in Fig. 5A2), though it decreased the cleavability of CCAmt Gag (percent density of p24; 68.9% versus 78.2% in Fig. 5B1; 45.3% versus 79.0% in Fig. 5B2; 41.9% versus 74.3% in Fig. 5B3), which was also parallel with viral replication data showing enhancement only in p17PRmt but not in CCAmt (Fig. 3). Considering together, the substitutions in C-terminal half of capsid compromised viral replication by impairing the Gag preprocessing, and NFV could counteract the negative impact only in the presence of the substitutions in N-terminal half of capsid. In the absence of the substitutions in N-terminal half of capsid, only partial counteraction was seen (Fig. 4d). In summary, NFV-induced viral replication enhancement of CL-4 was caused by two mechanisms; NFV facilitates the processing of Gag harboring the substitutions in the matrix and N-terminal half of capsid of CL-4, and NFV counteracts the impaired Gag cleavage caused by the substitutions in C-terminal half of capsid of CL-4 only in the presence of the substitutions in the matrix and N-terminal half of capsid of CL-4. Therefore, the full potential of the enhancement phenotype was achieved only with the cooperation of mutations in the entire Gag and protease region of CL-4.

Notably, we found several other PI-resistant isolates with the phenotype of PI-dependent replication enhancement (data not shown), suggesting that HIV-1 can evolve to acquire capability to replicate better with the drugs. Such replication enhancement with antiretroviral agents presents formidable challenge in the therapy of HIV-1 infection. Future studies of structural analyses of Gag precursor(s) harboring substitutions of these mutants are warranted to clarify the underlying mechanism(s).

Acknowledgments

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REVIEW ARTICLE

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Antiviral treatment of hepatitis C: present status and future prospects

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Abstract Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis. A substantial proportion of patients with chronic hepatitis C eventually develop hepatocellular carcinoma (HCC), which is one of the leading causes of death worldwide. Therefore, efficient antiviral treatments for HCV have long been needed. A recently developed combination therapy of pegylated interferon and ribavirin has dramatically improved the outcome of antiviral therapy for HCV infection. In genotype 1b HCV infection, 48 weeks of the combination therapy achieved eradication of the virus in 50% of patients, and in genotype 2 HCV infection, 24 weeks of the therapy resulted in viral eradication in 80%–90% of patients. By this eradication, an improvement in the hepatic fibrosis, an inhibition of HCC development, and an improvement in life expectancy were attained. Patients who did not respond to the combination therapy may be treated with long-term interferon monotherapy, which is not intended to eradicate HCV, but will lower the serum alanine aminotransferase (ALT) level. Thus, the treatment for HCV infection has progressed significantly, but therapies with new modalities, such as inhibitors of viral protease or RNA polymerase, are still being awaited.

Key words Hepatitis C · Interferon · Treatment

Introduction

Hepatitis viruses mainly infect the liver, causing hepatic diseases in humans. To date, five types of hepatitis virus, B, A, D, E, and C, have been found, in this order, and subjected to medical treatment. Hepatitis C virus (HCV) and hepatitis B virus (HBV) infections can develop into persistence, while hepatitis A virus and hepatitis E virus cause only transient infection. In Japan, chronic hepatitis caused by HCV infection currently poses the greatest problem because of the large number of patients affected and the high rate of patient mortality from complications, particularly hepatocellular carcinoma (HCC).[1]

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Chronic hepatitis C

It is estimated that there are approximately 170 million HCV carriers or patients with persistent HCV worldwide, and approximately 1.8 million patients in Japan. HCV infection occurs when blood contaminated with HCV enters the body directly. The infection routes include blood transfusion with HCV-contaminated blood products obtained a long time ago, sharing of needles among drug abusers, and the use of inappropriately disinfected acupuncture needles and tattoo needles, among others.[2] People undergoing folk remedies and hair-removal treatments should also be regarded as susceptible to HCV infection if these are invasive practices and nondisposable devices are used.

The problem with HCV infection resides in the very high rate of general HCV infections which are becoming chronic (approximately 70%). However, in the case of HCV infection via blood transfusion, the rate of reaching chronicity has been reported to reach 80%, probably because of a high virus load.

Virus markers of HCV infection required for the treatment of hepatitis

Some virus markers of HCV infection are available, as described below. Figure 1 shows a progress observation flowchart for anti-HCV antibody-positive patients obtained using these virus markers.

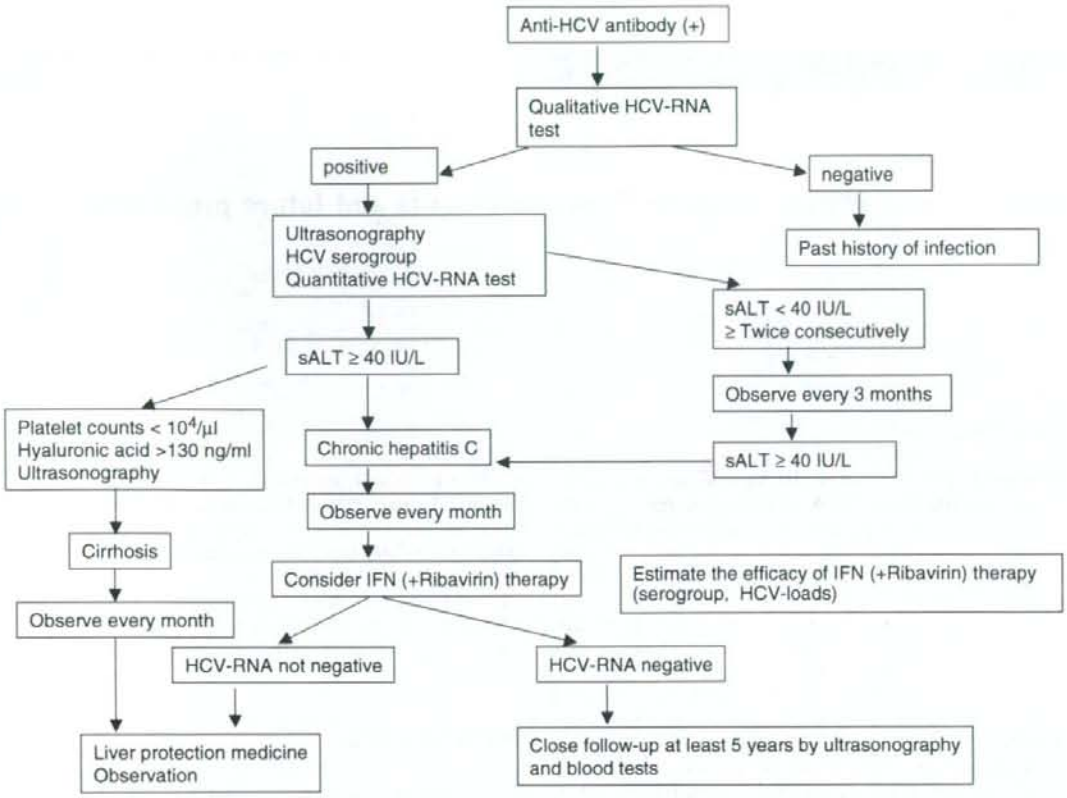

Fig. 1. Progress observation flow chart for anti-HCV antibody-positive patients. *HCV*, hepatitis C virus; *ALT*, alanine aminotransferase; *IFN*, interferon

Anti-HCV antibody

Anti-HCV antibody of low titer is frequently detected using sensitive HCV kits currently available in Japan. Patients with low anti-HCV antibody titers mostly have a history of remote HCV infection, while those with high titers generally have an ongoing infection. Hence, patients who test positive for anti-HCV antibodies are not necessarily infected with HCV at present. When the antibody titer is found to be low, a history of infection (i.e., currently cured) should be suspected. To verify this, a sensitive qualitative HCV–RNA measurement is required (reverse transcriptase–polymerase chain reaction (RT–PCR) method).

Meanwhile, it should be noted that during the early stage of HCV infection (2–3 months from the initial HCV exposure), patients do not test positive for anti-HCV antibody (window period).

HCV–RNA

To confirm the presence of HCV, we use an HCV–RNA assay by RT–PCR. There are two types of RT–PCR assay, a qualitative one and a quantitative one. However, the latter has a relatively low sensitivity. Therefore, the qualitative RT–PCR assay PCR is used to monitor the presence or absence of HCV, and hence the efficiency of an antiviral drug. For an estimation of the efficacy of antiviral treatment with interferon (IFN), a quantitative RT–PCR assay must be used.

Genotypes and serogroups of HCV

Many genotypes of HCV have been identified (i.e., there are HCV groups whose gene or genomic sequences differ to some extent). HCV genotypes are clinically important because the efficacy of IFN therapy varies depending on the HCV genotype. In Japan, the majority of HCV patients have HCV genotypes 1 or 2. Because the HCV genotype is determined on the basis of restriction fragment length polymorphism (RFLP) by PCR assay, the determination procedure is somewhat complicated. In order to determine the responsiveness of patients with chronic hepatitis C to IFN therapy easily (rapidly and accurately), serogroup (SG) identification by enzyme immunoassay is useful.[3] Patients

are classified as SG-1 (corresponding to HCV genotype 1) or SG-2 (corresponding to HCV genotype 2). Many patients classified as SG-1 are resistant to IFN, whereas many patients classified as SG-2 are generally responsive to IFN therapy.

Natural course of HCV infection

HCV patients commonly develop "acute hepatitis" 2 or 3 months after the initial exposure. However, many patients are unaware of this development because they have minor subjective symptoms and hardly exhibit jaundice. About 20% to 30% of patients exhibiting acute hepatitis recover spontaneously from the disease, but acute hepatitis develops into chronic hepatitis in the remaining 70% to 80% of patients (hepatitis persisting for more than 6 months is defined as chronic hepatitis). In general, these patients enter an "inactive phase" of hepatitis C, which persists for more than 10–15 years. The serum alanine aminotransferase (ALT) level, which indicates the extent of hepatocytic damage, is within the normal range during the inactive phase, but viral replication continues even during this period (Fig. 2).

Chronic hepatitis C frequently enters the "active phase" after an inactive phase of 10–15 years; however, this period varies greatly depending on the individual. In the active phase, the serum ALT level becomes approximately 2–3 times higher than the normal level. The problem with chronic hepatitis C is that it does not resolve spontaneously once it enters the active phase. If chronic hepatitis is left untreated, the risk of progression to cirrhosis increases without the patient realizing it. Thus, hepatitis C is characterized by its gradual but steady progression.[4]

With the progression to cirrhosis, there is an increasing risk of developing HCC. This risk has been reported to have an annual rate of 5% to 7%.[3] Ideally, HCV-infected patients should have the disease diagnosed during the inactive phase of chronic hepatitis so that, upon transition to the active phase, the patients can start receiving antiviral therapy for HCV.

Treatment of HCV infection

HCV infection is treated using mainly IFN preparations. These IFN preparations are outlined below in their order of development.

IFN monotherapy

IFN monotherapy was first introduced for the treatment of chronic hepatitis C. In Japan, the treatment of chronic hepatitis C generally starts with the daily administration of 6–10 million units of IFN for 2–4 weeks, followed by administration three times weekly for 6 months. In Europe and the USA, 3 million units of IFN are administered three times weekly from the start, and this is continued for a year. The efficacy of the therapy is evaluated after 6 months of IFN treatment. If an HCV-RNA test is negative by a qualitative RT-PCR assay at this time, it indicates that the patient obtained a sustained virological response (SVR) and is considered to be practically free of HCV.

IFN monotherapy had conventionally been used for non-A/non-B hepatitis from around 1985, prior to the discovery of HCV. A nationwide survey carried out by a research group supported by the former Ministry of Health and Welfare in 1995 showed that the overall SVR rate following IFN monotherapy for chronic hepatitis C (the administration of 6–10 million units per day) for 6 months was approximately 30%. SVR rates at facilities across Japan were nearly equal to this value. However, among patients with HCV genotype 1, who accounted for approximately 70% of all Japanese patients infected with HCV, and particularly those with a high viral load (defined as HCV-RNA >100 KIU/ml in Japan), a SVR was obtained in only 2% to 7% of cases; i.e., the efficacy of treatment by IFN

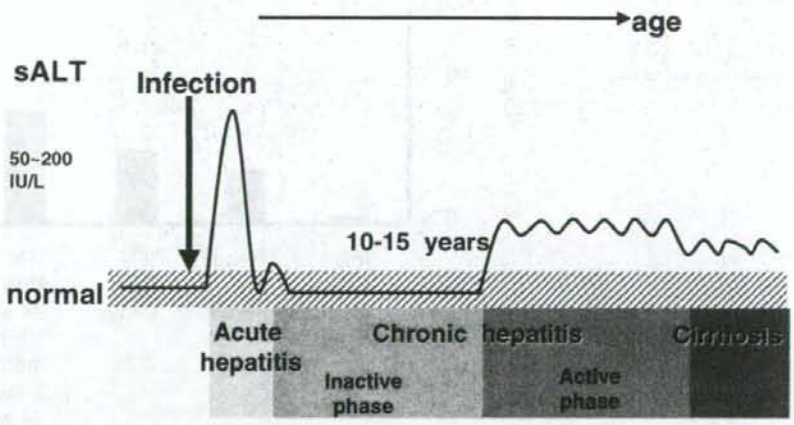

Fig. 2. Natural course of HCV-infected patients. Approximately 70% of acutely HCV-infected people develop persistent infection. After 10–15 years of the inactive phase, most chronic hepatitis C patients move into the active phase. One-third of chronic hepatitis C patients are assumed to develop cirrhosis. *sALT*, serum alanine aminotransferase

monotherapy was low. These patients with HCV genotype 1 at a high viral load have what is called "intractable hepatitis C."

IFN therapy in combination with ribavirin

IFN is also administered in combination with ribavirin, an antiviral drug. In Japan, the use of ribavirin was approved in December 2001. Ribavirin (600–800 mg daily, divided into two doses) is taken orally throughout the period of IFN injections. Ribavirin is a synthesized nucleic acid derivative and, when administered in combination with IFN, shows an increased antiviral activity.

In clinical studies of IFN therapy in combination with ribavirin conducted in Japan, a SVR rate of approximately 20% was obtained even in patients with HCV genotype 1 at a high viral load, i.e., "intractable hepatitis C," and who were less responsive to IFN monotherapy. Because patients on IFN monotherapy used as the control showed a SVR rate of only 2.3%, the concomitant use of ribavirin contributed to an approximately 10-fold increase in antiviral activity.[6]

The efficacy of IFN therapy in combination with ribavirin after its inclusion in the health insurance program is very similar to that found in a clinical study in Japan. However, the adverse effects of this combinational therapy have generally been more severe than those observed during the clinical study period. The drop-out rates of patients who could not complete the combinational therapy were as high as 15%–20%, and this led to a decrease in SVR rate calculated on intention-to-treat (ITT). In other words, the number of patients who dropped out of the treatment is added to the denominator. Adverse drug reactions that reduce the quality of life (QOL), such as hemolytic anemia, severe malaise, anorexia, and taste disorders, are frequently observed, particularly in many elderly patients. Indications for IFN therapy in combination with ribavirin should be considered carefully for patients aged 65 years or older.

Long-term interferon therapy

In cases of long-term IFN therapy, IFN is administered two or three times a week for a period of 2 years or more. The purpose of this therapy is not the eradication of HCV, but the normalization of serum ALT levels and eventually the suppression of HCC development. This is a promising therapy for patients who cannot be treated with ribavirin because of its adverse effects, or for those who were not able to continue with the combination therapy of IFN and ribavirin.

PEG–IFN therapy in combination with ribavirin

PEG–IFN is an interferon molecule covalently bonded to polyethylene glycol (PEG), which shows a sustained release. PEG–IFN characteristically requires subcutaneous administration only once weekly, as compared with the conventional type of IFN which requires administration three times a week. PEG–IFN therapy alone has a higher efficacy than the conventional IFN monotherapy, but it has been demonstrated that PEG–IFN therapy used in combination with ribavirin shows an even higher efficacy[7,8] (Fig. 3).

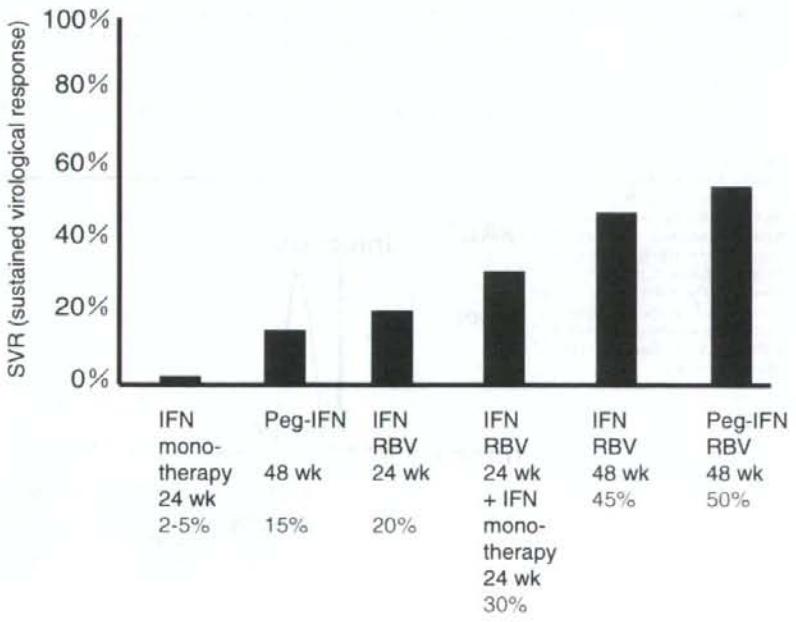

Fig. 3. Changes in anti-HCV therapy, including interferon for intractable (genotype 1b, high viral loads) chronic hepatitis C patients. After the introduction of IFN monotherapy for chronic hepatitis C, the efficacy of IFN therapy has gradually increased with the addition of ribavirin, the introduction of pegylated IFN, and an extension of the duration of therapy. *IFN*, interferon; *RBV*, ribavirin

PEG–IFN therapy in combination with ribavirin is expected to increase the SVR rate to approximately 50% even in patients infected with HCV genotype 1 at a high viral load, and to approximately 60% in all patients infected with HCV. The efficacy in those infected with genotype 2 HCV reaches 80%–90%. In Japan, treatment with PEG–IFN α-2a (Pegasys) alone was approved in December 2003. The combined use of PEG–IFN α-2b (PegIntron) and ribavirin (Rebetol) was also approved in December 2004. These treatments with PEG–IFN are generally administered for 48 consecutive weeks. Continuation of the treatments for 48 consecutive weeks is important, although it may be necessary to decrease the dose owing to adverse drug effects.

The adverse effects of PEG–IFN therapy in combination with ribavirin are almost the same as those of conventional IFN therapy. However, such adverse effects are generally minor (for example, fever), and the therapy requires administration only once per week, thereby improving the patient's QOL. Because there is the possibility of drug accumulation in the body and an associated exacerbation of adverse effects owing to the sustained-release formulation, very careful observation of patients is required. There have been reports of other problematic adverse effects of this combinational therapy compared with those of the conventional IFN preparation, e.g., decreased counts of leukocytes, and particularly of neutrophils. Some patients exhibit severe thrombocytopenia. It is mandatory to confirm neutrophil count immediately before every administration.

It is currently specified that PEG–IFN therapy used in combination with ribavirin is the best choice in the treatment of intractable hepatitis C of genotype 1 at a high viral load. This combinational therapy is thus administered first. It has recently been suggested that an extended administration period of 72 weeks for PEG–IFN therapy in combination with ribavirin proves effective in patients who are slow in showing a SVR.

Efficacy of antiviral therapy and its effect on patient prognosis

The following points have been reported in the literature: in patients in whom HCV was eradicated mainly by IFN monotherapy, hepatic fibrosis is improved,[9] the development of HCC is inhibited,[10] and life expectancy is also improved.[11] It has thus been indicated that if the eradication of HCV can be achieved, chronic hepatitis C prognosis is improved. It has also been reported that in patients in whom serum ALT level was normalized (even if this was transient), despite the failure to eradicate HCV (cases referred to as a biochemical response (BR)), the development of HCC was delayed in the short term. However, because no improvement in fibrosis was observed, it will probably be impossible in the long term to block the development of HCC. It has also been demonstrated that when curative treatment is carried out even after the development of HCC, subsequent IFN-based treatment could inhibit the recurrence of HCC.

Treatment of hepatitis C patients who do not respond sufficiently to IFN

Liver-protection therapy

Liver-protection therapy aims to delay the progression of chronic hepatitis by controlling inflammation in patients in whom HCV could not be eradicated. An ursodeoxycholic acid preparation (Urso) and a glycyrrhizin preparation (Stronger Neo Minophagen C) are used in combination as a liver-protection therapy. These drugs inhibit hepatic inflammation and decrease serum ALT level, but they do not decrease HCV load. It was reported that Stronger Neo Minophagen C delays the progression of chronic hepatitis and the onset of HCC.[12] The ursodeoxycholic acid preparation decreases serum ALT level, but its action of delaying the progression of chronic hepatitis has not yet been verified.

Hepatitis C generally progresses slowly and is less likely to aggravate rapidly, unlike hepatitis B, which may aggravate very rapidly, and progresses steadily. Liver-protection therapy, which retards the progression of the disease by controlling inflammation, can therefore be considered significant in hepatitis C. This therapy is applied mainly when it is impossible to use IFN due to its adverse effects, or when patients do not respond sufficiently to IFN therapy, including in combination with ribavirin. Liver-protection therapy is also administered as a temporary measure until a therapy in combination with IFN is started.

Phlebotomy

Iron deficiency leads to a decrease in serum ALT level, and its use as a therapy for chronic hepatitis C has begun to be appreciated. This is based on the observation that reactive oxygen species (ROS) production increases in hepatitis C patients, which leads to the development of liver disease and eventually HCC. Because intrahepatic iron plays an important role in ROS production (Fenton reaction), phlebotomy is designed to suppress ROS production by inducing intrahepatic iron deficiency. In fact, decreasing the serum ferritin level (an indicator of iron store) to 10 ng/ml or lower leads to a significant decrease in serum ALT level.[13] This is a promising therapy for patients who do not respond sufficiently to IFN therapy, or who are unable to receive it and do not respond to the above-mentioned liver-protection therapy either.

Conclusions

An overview of the current status of research on the progression of chronic hepatitis C and the treatment methods available has been presented and discussed in terms of the effects and limits of these methods. The early detection of HCV infection makes it possible to apply antiviral therapy at the appropriate time. It is particularly worth noting that

it has become possible for antiviral therapies to eradicate viruses in a majority of HCV patients, and to suppress and control the progression of HCV infection (or acute hepatitis C) to chronic hepatitis and subsequently to HCC. However, the limits of the current IFN-based therapies have also become evident. Specific antiviral drugs targeting HCV enzymes (including viral proteases, helicase, and RNA polymerase) have recently been developed. The development of one antiviral drug has advanced to phase II clinical trials as of 2006.

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LETTERS TO THE EDITOR

Anti-HCV agent, ribavirin, elevates the activity of clotting factor VII in patients with hemophilia: a possible mechanism of decreased events of bleeding in patients with hemophilia by ribavirin

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The combination therapy with ribavirin and interferon-α (IFN-α) has been reported to be more effective than IFN-α monotherapy for eradicating hepatitis C virus (HCV) [1,2], including patients with concomitant hemophilia [3]. We observed significant decreases in doses of clotting factors used for hemostatic therapy in hemophiliacs during ribavirin administration (e.g. 3780 units per month before ribavirin treatment and 1600 units per month during ribavirin on the average) [4]. In our hospital, 47 hemophilic patients who had been treated for chronic hepatitis C with IFN-α alone demonstrated no significant reduction in the use of clotting factor. This observation strongly suggests that the addition of ribavirin leads to the reduction of clotting factors used for bleeding in hemophiliacs. One suggestion comes from a case report that described an increase in warfarin dose requirement in a patient with heart valve prosthesis after starting this anti-HCV combination therapy [5].

These observations led us to investigate the ribavirin-induced change in vitamin K-dependent coagulation factors. To this purpose, we have measured the clotting activity of factor (F)VII, X, and prothrombin in hemophilic patients who were receiving the anti-HCV combination therapy. The protocol of therapy and analysis was approved by the Nagoya University institutional review board and written informed consent was obtained from each patient before treatment. Nine hemophilic patients, including seven hemophilia A and two hemophilia B (mean age ± SD: 42.5 ± 10.4 years old), whose characteristics were previously described [4], were entered in this study.

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The liver biopsy performed before starting the combination therapy did not show cirrhosis but chronic hepatitis in all patients analyzed. During this study, all patients were treated with the same 24-week regimen of IFN-α 2b (Intron A®, Schering Plough, K.K., Osaka, Japan) and oral ribavirin (600–800 mg day^{-1} of Rebetol; Schering-Plough, Kenilworth, NJ, USA). All statistical analyses were performed with STATA ver.7 software (STATA Corp., College Station, TX, USA) and the P-value < 0.05 was considered statistically significant.

The procoagulant activity of FVII in plasma has been elevated in all of nine ribavirin-treated hemophilic patients in comparison with that before ribavirin administration (Fig. 1A). The average and standard deviation for the elevation of FVII activity was 15.7% ± 8.8% ($P < 0.04$ in before vs. during ribavirin treatment; max. 28%; min. 5%). This elevation of FVII activity was independent of improvement of liver function (i.e. albumin, total billirubin, cholinesterase) in the patients (not shown). Only two patients, one has HIV infection and the other has hepatitis B virus concomitant with HCV, did not show a substantial elevation of FVII activity (i.e. 5% and 8%, respectively). We then measured activated FVII (FVIIa) levels in patients' plasma before and during ribavirin treatment using STACLOT® VIIa-rTF (Diagnostica Stago, Asnieres, France) [6] and observed substantial increases in FVIIa (e.g. 25.3 ± 14.8 mU mL^{-1}), which were almost compatible with elevation of FVII clotting activity. The plasma levels of FX and prothrombin were unchanged by ribavirin treatment in all of nine hemophilic patients (not shown). The elevation of FVII clotting activity by ribavirin is consistent with the previous observation of warfarin resistance in a ribavirin-treated patient [5].

To investigate the mechanism of ribavirin-induced elevation of FVII activity, we analyzed the gene expression of FVII in cultured normal human hepatocytes (Cambrex Bio Science Walkersville, Inc., Walkersville, MD, USA) or human hepatoma cell line, HepG2 cells (ATCC, Manassas, VA, USA),

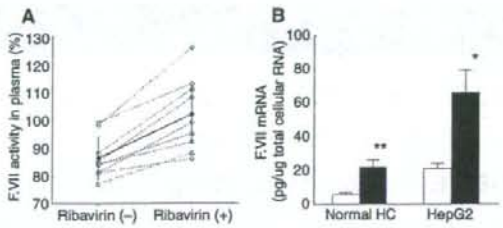

Fig. 1. Clotting activity of FVII in plasma of hemophilic patients and the mRNA expression of FVII in cultured human hepatocytes with or without ribavirin. (A) Each clotting activity of FVII in plasma of nine hemophilic patients before and at 4 weeks after starting ribavirin therapy was shown as open circle and dashed line, respectively. The average and SD of all patients was expressed as closed circle and error bar (without ribavirin: 86.3% ± 7.6%; with ribavirin: 102.0% ± 10.3%; $P < 0.04$). (B) Normal human hepatocytes or HepG2 cells had been cultured with (■) or without (□) ribavirin for 48 h. The mRNA expression of FVII was quantitated by real-time RT-PCR assay. Each value is expressed as the mean and SD from three sets of experiments. All of real-time RT-PCR assays were performed in duplicate. $*P < 0.02$; $**P < 0.01$.

which were cultured in medium with ribavirin at clinically therapeutic concentration (150 μg mL^{-1}) in the presence of IFN-α 2b (0.75 μg mL^{-1}; kindly provided by Schering Plough, K.K.). The expression level of mRNA for FVII, FX, and prothrombin, was determined by real-time quantitative RT-PCR with the ABI Prisms 7700 Sequence Detection (Perkin-Elmer Biosystems, Foster City, CA, USA) and SYBR Green PCR Kit (Perkin-Elmer Biosystems), according to the manufacturer's recommendations. The sequences of primer pairs used to quantify mRNA of the above genes were described in the NCBI Sequence Viewer. Variations in sample loading were assessed by measuring β-actin mRNA. Comparison of quantitative RT-PCR results between two groups was performed with the two-sample *t*-test. Welch's method was applied when variance between two groups was unequal (statistical significance: $P < 0.05$).

Significant induction of FVII mRNA was demonstrated in cultured normal hepatocytes (fourfold; $P < 0.01$) or HepG2 cells (threefold; $P < 0.02$) at 48 h after ribavirin treatment (Fig. 1B). No significant induction of mRNAs for FX and prothrombin was detected in ribavirin-treated cultured hepatocytes or HepG2 cells (not shown). In hepatocytes, ribavirin may stimulate to synthesize FVII by binding specifically to the promoter region of FVII gene (under current investigation).

It is possible that not only the induction of FVII but also changes in other coagulation factors during ribavirin therapy may be responsible for the decreased events of bleeding in hemophiliacs. However, the elevation of FVII activity in plasma could contribute most to the increased hemostatic potential in hemophilic patients because the cell-based tissue factor-activated FVII would play a central role in initiating coagulation and in activating platelets followed by large scale thrombin generation [7]. Clinically, recombinant activated FVII has been widely used as an antidote to control and prevent excessive hemorrhage in hemophilic patients with inhibitors [8]. Meanwhile, it was reported that even 10–20% of increase in plasma FVII/FVIIa would be an independent risk factor for coronary heart disease in healthy individuals [9,10], suggesting that a substantial elevation of endogenous FVII levels could result in an increased thrombotic potential. In general, the occurrence of spontaneous bleeding events in hemophiliacs is dependent on the critical hemostatic balance. In these conditions, 15–20% elevation of intrinsic FVII activity in plasma (Fig. 1A), because of the continuous induction of endogenous FVII by ribavirin (Fig. 1B), would contribute to the prevention from spontaneous bleeding in hemophiliacs. As a half-life of FVII in plasma is the shortest in all of coagulation factors, the continuous induction of FVII can maintain or increase the hemostatic value *in vivo*. If the prophylaxis to bleeding in hemophilic patients by ribavirin treatment were executable, it would result in much improvement of quality of their life and in large reduction of medical expenses in the country.

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5．血友病におけるHCV感染症の実態と新しい治療

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Summary C型慢性肝炎の治療法は現在，ペグインターフェロンα＋リバビリン治療が行われるようになり，genotype 1型の100 KIU/mL以上の高ウイルス量に対して著効率は約50%であり，genotype 1型高ウイルス量以外の症例では著効率は約90%得られるようになっている。血友病に合併したC型慢性肝炎に対しても同等の成績が得られているが，HIVを重複感染している場合には著効率はかなり低下する。肝硬変への進展および肝癌発症を抑制するためにはウイルス駆除が重要となり，治療効果と副作用などを十分検討して治療をする必要がある。

はじめに

血友病を含む凝固異常患者においては，1980年代中頃までに使用されていた非加熱凝固因子製剤により，大多数がC型肝炎ウイルス（hepatitis C virus：HCV）に感染しており，自然排除された場合もあるが，多くは持続感染している。また，HCVとHIV（human immunodeficiency virus）が重複持続感染している場合もある[1~4]。C型肝炎は約7割で肝炎が慢性化し，感染時期，アルコール摂取や性別など他の因子の影響にもよるが，その後およそ10～30年経過して肝硬変となる[5]。肝硬変になると門脈圧亢進症による食道静脈瘤，腹水や肝性脳症が生じてくる，また，肝癌が発生してくる。このため，QOLが悪化し，予後が悪くなる。血友病の場合もC型肝炎に感染していることで，肝疾患および肝癌で死亡する確率が一般人と比較して高い[4]。さらにHIVを重複感染している場合にはC型肝炎の進行が早く[6~11]，HCV単独感染の場合より予後がより悪い[12]。このことから血友病患者の将来的なQOLと予後を改善するには，HCVを排除することにより肝病変の進行を阻止することが重要となる。これにより，結果としてHCV関連死を減らすことが目標である。本稿では血友病におけるC型慢性肝炎の治療をHIV重複感染例も含めて，最近の治療法の

《略語一覧》
HCV（hepatitis C virus；C型肝炎ウイルス）　HIV（human immunodeficiency virus）

向上および問題点について概説する。

1．C型慢性肝炎の治療

HCVウイルスはインターフェロン（interferon：**IFN**）治療により排除が可能である。HCVの持続陰性化率（Sustained Virological Response：**SVR**）はHCVのgenotypeにより大きく異なるが，リバビリンの併用や**PEG-IFN α**（Pegylated interferon α）の登場といったIFN治療法の進歩にともない向上している。

リバビリンは，プリン骨格をもつ合成核酸アナログで抗ウイルス活性をもつ。リバビリン単独では**ALT**（alanine aminotransferase）値の低下はみられるもののウイルス排除には至らないが[13]，欧米でIFN α単独療法とIFN αとリバビリンの併用療法の大規模な比較試験が行われ，併用療法はIFN α単独療法と比較して，SVR率を有意に改善すると報告された[14]。

PEG-IFN αはIFN αにポリエチレングリコール（polyethyleneglycol：**PEG**）を結合させたものであり，体内から緩徐に排泄され，血中濃度を維持できるため，週1回の投与で効果が持続され，発熱などの症状は従来のIFN週3回投与時と比較して軽くなっている。これにより，治療効果も従来のIFN α単独と比較してPEG-IFN αでSVR率の向上が認められている[15]。その後，PEG-IFN αとリバビリンの併用療法ではSVR率のさらなる向上が認められている[16]。

図1はC型慢性肝炎におけるウイルス排除の効果に重要な因子となるHCV genotype別の治療の進歩を示したものである[17]。IFN α（α 2aあるいは2b）単独療法での著効率はgenotype 1，4，5，6では，週3回3MUを24週間投与した場合には5％であり，48週投与では15％であった。一方，genotype2，3ではそれぞれ20％，40％であった。IFN（週3回3MU）とリバビリン（1,000～1,200 mg/日）の24週あるいは48週投与ではgenotype 1，4，5，6で35％，genotype 2，3では80％と向上しており，さらにPEG-IFN αとリバビリンの併用48週投与では，genotype 1，4，5，6で48％，genotype 2，3では88％のSVR率が得られている。

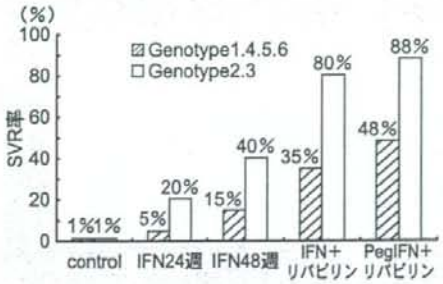

図1　Genotype別C型慢性肝炎治療効果
（文献17より引用）

本邦においては，従来IFN（週3回6MU）24週間投与がなされてきたが，2001年12月にIFN αとリバビリンの併用療法が保険適応となり，2002年2月にはIFN αの長期投与が可能となり，本邦での成績も**図1**の成績とほぼ同様である。2003年12月にはPEG-IFN α-2aの単独投与，2004年12月にはgenotype1かつ高ウイルス量例に対してPEG-IFN α-2bとリバビリンの併用48週間投与が，また2005年12月にはgenotype 1型高ウイルス量以外に対してPEG-IFN α-2bとリバビリンの併用24週間投与が認可された。PEG-IFN α-2bとリバビリンの併用療

《略語一覧》
IFN（interferon；インターフェロン）
PEG-IFN α（Pegylated interferon α）
PEG（polyethyleneglycol；ポリエチレングリコール）
SVR（Sustained Virological Response；持続陰性化率）
ALT（alanine aminotransferase）

表1　ALT持続正常例に対するIFN療法

Author	N	Suhedule used	SVR (%)	ALT elevations
Marcellin et al., 1997	Review	6～12 months IFN monotherapy	13	～50%
Rossini et al., 1997	19	3MU IFN tiw×12months	10	26%
Tassopoulos et al., 2002	37	5MU IFN tiw×6months	21.6	8%
Sakugawa et al., 2003	19	6～10MU IFN tiw×6months	42	n.r.
Mamori et al., 2004	18	6～10MU IFN daily/tiw×6months	38.9	33%
Duvryak et al., 2003	12	3MU IFN tiw＋1,000 Riba daily×6months	42	No
Erhardt et al., 2003	20	3MU IFN tiw＋1,000～1,200 Riba daily×12months	40	No
Hui et al., 2003	52	3MU IFN tiw＋1,000～1,200 Riba daily×12months	38.5	9.40%
Mangia et al., 2004	35	3MU IFN tiw＋1,000 Riba daily×6months	62.8	18%
Lee et al., 2001	19	3～5MU IFN tiw＋1,000 Riba daily×12months	47	No
Hasau et al., 2001	32	4～5MU IFN tiw＋1,000 Riba daily×12months	28	No
Jacobson et al., 2004	56	3～5MU IFN tiw＋1,000 Riba daily×12months	32	8%
Zeuzem et al., 2004	210	PegIFN weekly＋800 Riba daily×12months	52	11%

（文献20，21より引用）

法の国内臨床開発試験によるSVR率は，genotype1かつ高ウイルス量症例では47.6%，genotype1かつ高ウイルス量以外の症例では87.3%であり，欧米の成績と同等のSVR率が得られている。しかしながら，国内臨床開発試験によるとgenotype1高ウイルス量の難治例では12週の時点でHCV RNAの陰性化がみられず，24週までにHCV RNAが陰性化した場合には，48週の治療では36．4%のSVR率しかえられていない。これに対して，海外のデータではあるが，PEG-IFN＋リバビリン治療にて12週でHCV RNAが陰性化せず24週で陰性化した場合に，延長して72週投与をした場合のほうがSVR率は高かった[18]。また，PEG-IFN＋リバビリン治療により4週の時点で陰性化しなかったC型慢性肝炎患者において，48週投与と72週投与にrandomizeし比較したところ，genotype1の患者においてSVR率は72週投与で44%と，48週投与の28%と比較して有意に高率であった[19]。したがって，HCV RNAの陰性化の遅い症例では48週をさらに延長して72週投与したほうが，著効率が上昇する。

また，HCV感染者のうち約30%程度が正常ALT値を示し，そのうち70%ぐらいが6～12カ月後の検査でも正常値のままであり，持続ALT正常例である。持続ALT正常例に対しての治療はIFN単独療法の時代にはSVR率も低く，治療後にALT値が上昇する割合が高かったが，IFN＋リバビリン治療ではSVR率も高くなり，問題となる治療後のALT上昇もあまり高くないことが示されている（**表1**）[20, 21]。さらに，PEG-IFN＋リバビリン治療においても，ALT正常例に対する治療でSVR率は52%と，ALT異常例での治療成績とほぼ同等であり，ALT上昇もあまりみられていない[21]。ALTの正常値は現在各施設の基準値で判断されているが，ALT値正常例においても肝生検を行うと肝の線維化が進行している場合があり，ALTの正常値の設定が問題となる。男性＜30 IU/L，女性＜19 IU/Lが本来の正常値であるとの報告[22]もあり，またALT正常例に対してPEG-IFNリバビリン併用療法を行いウイルスが低下した場合に，ALT値は投与前よりさらに低下

することからも，現在の正常値の基準を再考する必要がある。SVRが得られなくても，治療終了後しばらくALT値が正常を持続する，すなわち生化学的著効が得られる場合もあり，その際もC型慢性肝炎の進行を遅らせ肝癌の発生も遅らせる効果が期待できる。

① リバビリン併用療法によりHCVウイルス排除ができなかった，② 重篤な疾患がありIFNあるいはリバビリンを使用できない，③ 肝病変の進行により白血球や血小板などがすでに低下していてIFN治療ができない，④ 貧血がある，あるいは出産の予定がありリバビリンの併用が行いにくい。上記①から④の場合には肝庇護療法にて肝炎を鎮静化し，肝発癌を抑制することが重要である。強力ネオミノファーゲンシー®の長期投与によりALTを低下させることで，肝炎の沈静化が得られ，発癌率を低下させる効果がある[23]。以前はC型慢性肝炎に鉄摂取を勧められていたが，C型慢性肝炎では肝に鉄が沈着しやすく，肝障害の一因となっている。定期的に200 mLから400 mLの瀉血をおこない血清フェリチン値を10 ng/mL以下にすることによりALT値が改善すると報告され[24]，2006年から本邦でもC型慢性肝炎に対して瀉血療法が保険適応となっている。

2．血友病におけるC型慢性肝炎の治療

C型慢性肝炎単独感染の場合との違いは，血友病ではHCVウイルスの感染時期が早期で繰り返しHCVに暴露していること，また肝生検時に出血の危険性が高いことが問題となる。C型肝炎の感染時期と肝炎の進行については，血友病でHIV陰性のC型慢性肝炎患者では感染から肝硬変までの経過は平均21.6年であり，血友病のないC型慢性肝炎単独感染の場合と差がないと報告されている[1]。すなわち，血友病においてはC型慢性肝炎にHIVが重複するかどうかで肝炎の進行が違ってくる。しかし，一般には若年で感染した場合は高齢で感染した場合と比較して進行が遅いと報告されており[25]，血友病では若年で感染しているわりには進行が早いとも考えられ，繰り返し暴露していることも影響しているかもしれない。

肝生検は肝疾患の重症度を評価するのに"gold standard"であり，HCV患者の予後や治療戦略を立てるのに重要である。血友病において肝生検をする場合には，施行前後に必要な凝固製剤を補充することで出血のリスクを軽減させる必要がある[26]。最近では採血のデータにより肝線維化を評価したり[27]，FibroScanという非侵襲的な肝線維化定量法としてトランジエンド・エラストグラフィー技術を使用して，より肝生検のデータに近づく試みがなされている[28]。これらのデータが肝生検の結果に近いデータが出るようになれば，特に血友病患者に対しては恩恵があると考えられる。血友病において，全症例の約70%がgenotype 1型の113名の患者に対するIFN単独療法，IFN＋リバビリン併用療法とのrandomise試験で，それぞれSVRが7%と29%と，有意にリバビリン併用療法で治療効果が高かった[29]，また，PEG-IFN＋リバビリン治療により，6カ月の投与でgenotype2.3では82%，12カ月の投与でgenotype1.4では38%にSVRが見られたと報告されている[30]，このようにC型慢性肝炎の治療成績とほぼ同等であることが報告されており[31, 32]，肝硬変，肝癌への進展を回避するためにウイルスの排除をすべきである。しかしながら，ウイルス排除ができなかった場合には，出血および静脈投与する血管の状態が問題とはなるが，強力ネオミノファーゲンシー®投与や瀉血療法をC型慢性肝炎治療の場合と同様に行い，ALT値をできるだけ正常値にコントロールすることが大切である。

表2　HIV重複感染C型慢性肝炎に対する治療効果

Number	study	Journal (文献No.)	IFN＋リバビリン			PegIFN＋リバビリン		
412	RIBAVIC	JAMA (37)	Genotype1.4	Genotype2.3.5	overall	Genotype1.4	Genotype2.3.5	overall
		SVR (%)	6	43	20	17	44	27
868	APRICOT	NEJM (38)	Genotype1	Genotype2.3	overall	Genotype1	Genotype2.3	overall
		SVR (%)	7	20	12	29	62	40
133	ACTG5071	NEJM (39)	Genotype1	non-Genotype1	overall	Genotype1	non-Genotype1	overall
		SVR (%)	6	33	12	14	73	27
95	Barcelona	AIDS (40)	Genotype1.4	Genotype2.3	overall	Genotype1.4	Genotype2.3	overall
		SVR (%)	7	47	21	38	53	44

(文献37～40より引用)

3．HIVを重複感染している血友病患者の治療

C型肝炎感染の血友病患者の一部には，HIVを重複感染している患者がいる。HIVが重複感染していると，C型肝炎単独感染の場合と比較して進行が早いことが報告されている[9, 11]。HAART (Highly Active Anti-Retroviral Therapy) 療法の向上で，HIV陽性の患者の予後は改善され，現在ではC型肝炎に関連した合併症による死亡が増加しており[33]，HCVウイルスの排除が重要となってきている。HIV重複感染によりHCV RNA量が高値の症例が多いこともあるが，欧米でのIFN単独療法によるSVR率はC型慢性肝炎単独感染でのSVR率と比較し，やや低値である報告が多い。日本においては，IFN単独療法ではHIV重複感染のC型慢性肝炎に対しては治療効果が低かった[34]。リバビリンの併用療法により，HIV重複感染者においても低いながらSVRが得られるようになった。しかし，リバビリンを併用することにより新たな副作用がみられるようになった。すなわち，致死的な乳酸アシドーシス[35]や膵炎，AZT（ジドブジン）併用による貧血の増加である[36]。このため，治療中は血清乳酸値やアミラーゼなどを測定して全身状態の十分な観察をしていく必要がある。HIV合併C型慢性肝炎に対するPEG-IFN＋リバビリン治療の4つの大規模試験によると，overallでのSVR率はIFN＋リバビリン治療で12～21%であったのに対して，PEG-IFN＋リバビリン治療では27～44%と治療効果が向上したが[37～40]，HCV単独感染者に対するPEG-IFN＋リバビリン治療の成績と比較するとSVR率は低値である。多変量解析によるSVRに寄与する因子は，genotypeが1型でない，HCV RNA量が800,000 KIU/mL以下と高ウイルスでないことであった。

表2にgenotype 1型とgenotype 1型以外に分けた治療成績をしめす。どのstudyでも著効率はC型慢性肝炎単独感染と比較して低値である。これは治療前のHCV RNAが高値であること，HIV重複感染の場合にはHCV RNAの消失率が低いこと，C型慢性肝炎単独感染の場合での中止率が10～15%であるのに対して，HIV重複感染の場合には20～40%と高率であることなどが考

《略語一覧》
HAART (Highly Active Anti-Retroviral Therapy)　　AZT (ジドブジン)

えられるが，他に抗HIV治療薬との相互作用なども考えられる。また，治療中のSVRの予測としてHCV RNAが12週時点で2 logの減少がみられない場合には，48週治療では98%の確率でSVRが得られないとしている。現時点で著効率を上昇させるには，ALT正常の早期の時点で治療を開始することや，副作用の問題点はあるが72週投与など治療を延長することでSVR率を向上させることが検討される。

4．新しい治療法

1）リバビリン類似薬剤

Viramidineは生体内でリバビリンに転換されるプロドラッグであり，赤血球への取り込みが少ないため，溶血性貧血を起こしにくいとされている。Phase Ⅲ試験ではPEG-IFNとの併用中に10 g/dL以下の貧血を起こした症例は，リバビリンの24%と比較して5%にしかみられず，貧血がある症例で有効となる可能性がある。

2）IFN類似薬剤

Albuferonはリコンビナントヒト血清アルブミンに融合するリコンビナントヒトIFN-α-2bからなる85.7-kDのタンパクで，半減期が長く，PEG-IFNより効果が長く，Phase Ⅱ試験ではgenotype 1型の初回治療例に対して2週に一度の900 μgの投与により，4週の時点で投与前と比較して3.3 log低下しており，期待される[41]。

3）HCV増殖に関連する酵素の阻害剤

HCV増殖に関連する酵素（ポリメラーゼ，ヘリカーゼ，プロテアーゼ，NS5B RNA依存性RNAポリメラーゼ）の阻害剤が開発されているが，なかでもNS3プロテアーゼ阻害剤であるSCH503034はPhase1b試験が終了した段階ではあるが，genotype 1型のPEG-IFN無効例に対してPEG-IFN α-2bとSCH503034 400 mgの併用療法で，最大約3 log程度低下したことが報告されており期待される。

Genotype 2型，あるいはgenotype 1型低ウイルス量のC型慢性肝炎であれば，PEG-IFN＋リバビリン治療での著効率は高く，治療を行うメリットが大きい。ウイルス排除ができれば肝硬変への進展抑制，発癌の阻止ができ，結果として肝疾患関連死を減らすことにつながるためである。しかしながら，genotype 1型の高ウイルス量ではまだ治療効果が低く，特にHIV重複感染に対しては副作用を減らし著効率を向上させる工夫，あるいは新しい治療法が必要である。

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