HCC in patients with low platelet counts, even when the ALT values fall within the current normal range.

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HEPATOLOGY

Prevalence and clinical characterization of patients with acute hepatitis B induced by lamivudine-resistant strains

Kazuhiko Hayashi,* Yoshiaki Katano,* Masatoshi Ishigami,* Akihiro Itoh,* Yoshiki Hirooka,* Isao Nakano,* Kentaro Yoshioka,† Motoyoshi Yano,‡ Hidenori Toyoda,§ Takashi Kumada§ and Hidemi Goto*

*Department of Gastroenterology, Nagoya University Graduate School of Medicine, Nagoya, †Division of Liver and Biliary Diseases, Department of Internal Medicine, Fujita Health University, Toyoake, ‡Department of Gastroenterology, Yokkaichi Municipal Hospital, Yokkaichi, and §Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan

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acute hepatitis, genotype, hepatitis B virus, lamivudine.

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Correspondence
Yoshiaki Katano, Department of Gastroenterology, Nagoya University Graduate School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466-8550, Japan. Email: ykatano@med.nagoya-u.ac.jp

Abstract

Background and Aims: Acute hepatitis caused by lamivudine (LMV)-resistant strains has not been reported, and the clinical impact of LMV-resistant strains on acute hepatitis is not known. The aim of this study was to investigate the molecular and clinical characteristics of patients with acute hepatitis B caused by LMV-resistant strains.

Methods: Forty-five patients with acute hepatitis B were studied. Hepatitis B virus (HBV) subgenotypes and LMV-resistance mutations were determined by direct sequencing of the preS and polymerase regions, respectively.

Results: HBV subgenotypes A2 ($n = 18$), B1 ($n = 1$), B2 ($n = 3$), B3 ($n = 2$), C1 ($n = 1$), C2 ($n = 19$) and C6 ($n = 1$) were detected in patients with acute hepatitis. LMV-resistance mutations were detected in two patients. LMV-resistance mutations (L180M, M204I) were detected in a patient with subgenotype C2 who had acute self-limited hepatitis. The other patient with LMV-resistance mutations (L180M, M204V) was infected with subgenotype A2 and had severe hepatitis.

Conclusion: LMV-resistant strains are rare, but they are starting to be found in patients with acute hepatitis B. Surveillance for detecting drug-resistant HBV strains would be important for clinical practice.

Introduction

Approximately 350 million people worldwide are infected with hepatitis B virus (HBV).[1] HBV infection causes a variety of clinical courses, such as self-limited acute hepatitis, fulminant hepatic failure, chronic hepatitis, and progression to cirrhosis and hepatocellular carcinoma.[2] Therefore, HBV infection is one of the most important global health problems. Most countries have performed universal vaccination to prevent HBV infection, but only high-risk groups, such as health-care workers and household contacts of HBV carriers, have received HBV vaccination in Japan.[3] Therefore, acute hepatitis is still a major problem in Japan. The frequencies of HBV strains that are rare in Japan have increased among Japanese patients with acute hepatitis B.[4-6] The distributions of the HBV strains in acute hepatitis are variable due to the changing social environment. Along the same lines, a study investigated acute hepatitis B induced by lamivudine (LMV)-resistant HBV strains, but acute hepatitis caused by an LMV-resistant strain has not been found, and the clinical impact of LMV-resistant strains on acute hepatitis is still unknown.[7] Surveillance of HBV strains associated with acute hepatitis B has been continued, and LMV-resistant strains have begun to be detected in patients with acute hepatitis B. Thus, the present study reports the clinical characteristics of patients in Japan with acute hepatitis B caused by LMV-resistant HBV strains.

Materials

Forty-five Japanese patients with acute hepatitis B who were treated at Nagoya University Hospital, Ogaki Municipal Hospital, Tosei Hospital, Yokkaichi Hospital, and Fujita Health University Hospital were enrolled in this study between January 2006 and September 2008. The patients were 37 men and eight women, with a mean age of 38.6 ± 12.9 years (range, 18–84 years). There were no patients who had received HBV vaccine. Acute hepatitis B was diagnosed as follows. Each patient had high titers of hepatitis B surface antigen (HBsAg) and immunoglobulin (Ig)M class antibody against HBV core antigen, elevated serum levels of alanine aminotransferase and absence of antibodies against other causative viruses, such as hepatitis A virus, hepatitis C virus, Epstein–Barr virus and cytomegalovirus. It was necessary to discriminate

between initial HBV infection and acute onset or reactivation of chronic HBV infection. Thus, serum HBsAg levels noted in previous medical records, blood donation screening, labor and delivery screening, or employment health screening, were obtained or were followed until negative of HBsAg and/or positive of hepatitis B surface antibody (HBsAb). No patients were using chemotherapeutic and immune modulating agents involved in HBV reactivation. Informed consent was obtained from all patients, and the study was carried out in accordance with the 1975 Helsinki Declaration. Serum was stored at –80°C for virological examinations.

Assay methodology

Hepatitis B virus DNA was isolated from peripheral blood with a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Nested polymerase chain reaction (PCR) analysis and direct sequencing of the preS, polymerase and precore/core regions were performed as reported previously.[7] In brief, each 50-uL PCR reaction contained 100 nM each primer, 1 ng template DNA, 5 uL GeneAmp 10× PCR buffer, 2 uL deoxyribonucleotide triphosphate and 1.25 U AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). Primers were: preS region sense 5′-TCACCTATTCT TGGGAACAAGA-3′ and antisense 5′-GGCACTAGTAAACTG AGCCA-3′; polymerase region, sense 5′-CCTGCTGGTGGCT CCAGTTC-3′ and antisense 5′-GGTTGAGTCAGCAAACAC ACTTG-3′; and precore/core region, sense 5′-ATGTCGACAA CCGACCTTGA-3′ and antisense 5′-GTATGGTGAGGTGAAC AATG-3′. Amplification conditions consisted of 5 min at 94°C followed by 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). The second PCR was done in the same reaction buffer with the first-round PCR product as template and the following sets of primers: preS region, sense 5′-TCACCTATTCTT GGGAACAAGA-3′ and antisense 5′-AGAAGATGAGGCATAG CAGC-3′; and polymerase region, sense 5′-GGATGTGTCTGC GGCGTTT-3′ and antisense 5′-ACCCCATCTTTTTGTTTTG TTAGG-3′. PCR products were detected by electrophoresis on 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers with a dye terminator sequencing kit (BigDye Terminator ver. 1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems). The neighbor-joining method[8] was used for phylogenetic analysis of the preS region to identify HBV subgenotypes. The bootstrap test with 1000 replicates was performed to confirm the reliability of the phylogenetic tree.[9]

Results

The results of the phylogenetic analyses of HBV subgenotypes of the 41 patients are shown in Figure 1. The HBV subgenotypes A2 ($n = 18$), B1 ($n = 1$), B2 ($n = 3$), B3 ($n = 2$), C1 ($n = 1$), C2 ($n = 19$) and C6 ($n = 1$) were detected. The prevalence of subgenotype A2 was increased, as previously reported. LMV resistance-associated mutations were detected within the HBV polymerase region (positions 116–214) by direct sequencing. Alignment of the amino acid sequence of the HBV polymerase region with LMV resistance-associated mutations was analyzed, and LMV-associated mutations could be detected in two patients at acute hepatitis onset. LMV-resistance mutations (L180M, M204I) were detected in a patient with subgenotype C2. The other patient with subgenotype A2 had LMV-resistance mutations (L180M, M204V). There were no resistant HBV mutants for other nucleoside/nucleotide analogs such as V173L, L180M or M204V/I. The clinical and virological characteristics of patients with LMV-resistant HBV strains are summarized in Table 1.

Discussion

Hepatitis B virus reverse transcriptase is an error-prone enzyme without proofreading capacity, and it is easy for frequent mutations to occur during viral replication. As a result, there are many well-known mutations that are associated with the pathogenesis of HBV infection.[10] LMV-resistant strains that have mutations in the polymerase region are induced by long-term administration of LMV.[11,12] LMV had been used widely for treatment for chronic hepatitis B and was available from 2000 in Japan. LMV-resistant strains have emerged in patients with chronic hepatitis. However, the prevalence and clinical impact of LMV-resistant strains in patients with acute hepatitis B are unknown. Thus, surveillance of LMV-resistant strains associated with acute hepatitis B had been conducted, but LMV-resistant strains could not be detected in 2006.[7] The possibility of acute hepatitis B caused by LMV-resistant strains exists, and the surveillance has continued. Of 45 patients with acute hepatitis, two were found to have LMV-associated mutations. We previously hypothesized that LMV-resistant strains may not have enough power to cause acute hepatitis. However, the present study demonstrated that LMV-resistant strains would have infectivity and would be capable of causing acute hepatitis. Less opportunity for infection may explain why previous studies failed to find acute hepatitis caused by LMV-resistant strains.

The infectious source of the LMV-resistant strains could not be confirmed. The subgenotypes of the patients infected with LMV-resistant strains were subgenotype A1 and C2, respectively. The patient infected with subgenotype C2 plus LMV-resistant strain had a history of sex with a prostitute 1 month before admission. Subgenotype C2 was the predominant subgenotype found in Japanese patients with chronic hepatitis B.[7,13–15] The infectious source would be a chronic hepatitis patient who developed resistant HBV mutants during long-term LMV treatment. The route of infection for the other patient with subgenotype A2 was unknown. HBV subgenotype A2 has been rarely reported in Japanese patients with chronic hepatitis B. However, subgenotype A2 has been increasing and has become responsible for the majority of patients with acute hepatitis B.[4,7,16] This study also confirmed that HBV subgenotype A2 has become widespread among Japanese patients with acute hepatitis. However, the origin of subgenotype A2 with an LMV-resistant mutation is not clear. The possibility of it coming from a patient with chronic hepatitis B is low, because subgenotype A2 is rarely found in Japanese patients with chronic hepatitis B who receive long-term LMV treatment. The other possible infectious source is a patient co-infected with HIV. Nucleoside/nucleotide analogs (NA) such as LMV were effective for both HBV and HIV. NA were used not only for treatment of HBV but also for treatment of HIV, and LMV-resistant strains have been reported.[17] HBV genotype A and HIV co-infection have been found among male

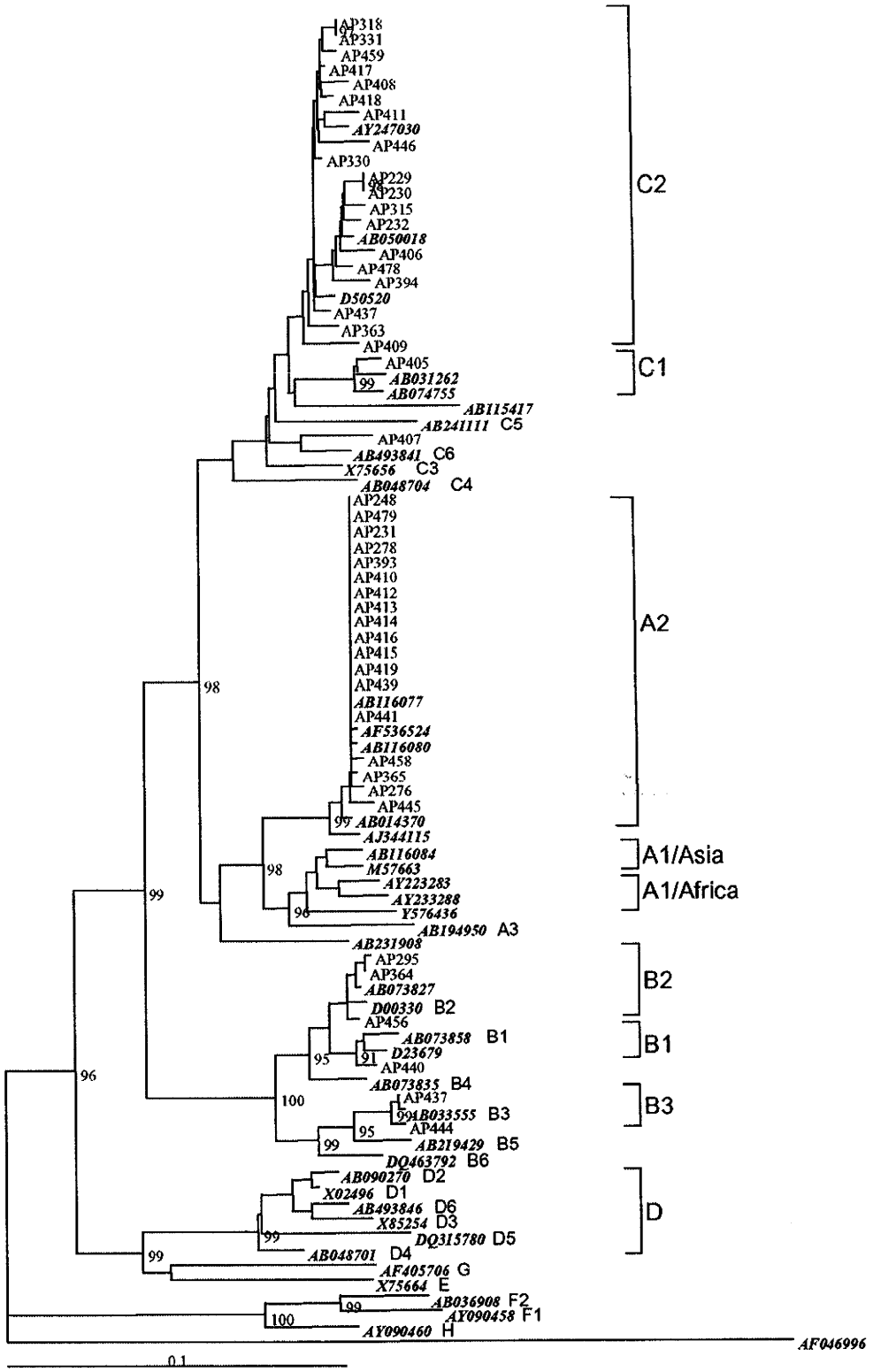

Figure 1 Results of phylogenetic analysis of 45 sequences from the preS region of hepatitis B virus (HBV) of acute hepatitis patients and 42 reference strains from a database and shown by accession number. Strains isolated from patients with acute hepatitis are indicated as AP. Phylogenetic analysis was performed by the neighbor-joining method with Woolly monkey HBV (AF046996) as out-group. Percentages of bootstrap values greater than 90% are shown on the nodes. The scale bar indicates genetic distance.

Table 1 Clinical characteristics

	Case 1	Case 2
Age (years)	32	32
Sex	Male	Male
ALT (IU/L)	4429	2820
AST (IU/L)	2709	1620
T Bil (mg/dL)	3.0	4.1
HBeAg	Positive	Positive
HBV (log copies/mL)	5.2	7.4
BCP1762/1764	T/A	A/G
PC1896	G	G
Route	STD	Unknown
Subgenotype	C2	A2

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; STD, sexually transmitted disease; T Bil, total bilirubin.

patients who have sex with men in Japan.[18] Because the patient infected with subgenotype A2 that was LMV-resistant was not co-infected with HIV, this was also inconclusive. The other possibility was that the infectious source could have been a foreign patient with subgenotype A2 in whom an LMV-resistant strain emerged. This study has the following limitations: a small number of patients, patients without symptom were not recruited, the identification of the infectious source. Thus, further studies such as a nationwide survey including blood banks to investigate asymptomatic patients, the need to make conclusion of the prevalence of patients with acute hepatitis B induced by LMV-resistant strains in Japan.

The patient with LMV-resistant mutations with subgenotype C2 developed self-limited hepatitis, while the other patient with LMV-resistant mutations with subgenotype A1 developed severe acute hepatitis. Basal core promoter (BCP) and precore (PC) variants have been shown to be associated with the severity of the clinical course of acute hepatitis. In particular, mutations at BCP/PC of HBV subgenotype C2 and B1 can increase the risk of progression to fulminant hepatic failure. The clinical impacts of basal core promoter and precore variants in other genotypes are unclear.[7,16] In the present study, both patients with acute hepatitis caused by LMV-resistant strains had wild-type BCP/PC variants. The wild-type BCP/PC variants were linked with mild self-limited hepatitis in the patient with subgenotype C2. The clinical impact of LMV-resistant strains on acute hepatitis appears to be not serious for subgenotype C2. Meanwhile, the mutations in the BCP/PC regions were not associated with the severity of acute hepatitis in the patient with subgenotype A2. Therefore, LMV-resistant mutations in subgenotype A2 might be associated with the severity of the clinical course. However, the present sample size was too small to allow evaluation of the clinical course in acute hepatitis B with LMV-resistant strains and to determine whether LMV-resistant strains have different effects on each subgenotype. Further studies are needed to clarify the influence of LMV-resistant strains on the clinical course of acute hepatitis B.

Lamivudine has begun to be used to treat patients with acute hepatitis to prevent progression to fulminant hepatic failure or chronic hepatitis. Some reports have shown the safety and effectiveness of LMV for the treatment of acute hepatitis B.[19,20] However, one clinical study that has been published did not confirm its efficacy.[21] Thus, the administration of LMV in acute hepatitis B is controversial. The use of LMV for all acute hepatitis was not of benefit and was not recommended for use in all patients. However, selected patients who have a high risk for progression to fulminant hepatic failure and chronic infection may benefit from LMV to prevent disease progression. There is a small possibility that acute hepatitis B can be caused by LMV-resistant strains, but previous studies did not consider LMV-resistant strains before they started to use LMV. Caution must be exercised when determining whether LMV should be used to treat acute hepatitis B because of the possibility of the development of LMV-resistant strains. In the present study, the patient with LMV-resistant mutations who progressed to severe hepatitis was treated with LMV and steroid. Despite the limited efficacy of LMV in suppressing viral replication of LMV-resistant strains, this patient recovered from severe acute hepatitis. Patients with severe acute hepatitis have a high risk for progression to fatal liver failure. However, patients not treated with LMV may have a full recovery and not progress to fulminant liver failure, either because of the efficacy of other treatment, such as steroid, or because the patients' immune reaction could clear the HBV infection. It is difficult to judge the clinical role of LMV-resistant strains in acute hepatitis based on this case. The present study included insufficient information about the magnitude of screening for LMV-resistant strains in acute hepatitis.

Lamivudine is associated with a high incidence of resistance.[22] Thus, the first-line agent for HBV infection has been changed from LMV to adefovir or entecavir because of their powerful antiviral effect and the lower likelihood of drug resistance mutations emerging. The emergence of drug resistance during long-term adefovir or entecavir therapy in chronic hepatitis B was not frequent compared to that with LMV.[23,24] With adefovir or entecavir, the incidence of LMV-resistant strains would be remarkably decreased, but the risk for other HBV drug-resistant strains still remains. Clinical use of anti-HBV agents such as adefovir, entecavir, telbivudine, clevudine and tenofovir has started, and multiple anti-HBV drug-resistant strains could occur in patients undergoing long-term treatment in the near future. Therefore, maintaining surveillance to detect drug-resistant strains of HBV may have a small impact, but it is important for clinical practice.

In conclusion, LMV-resistant mutations were previously rare but now appear to be prevalent among patients in Japan with acute hepatitis B. LMV-resistant strains must be considered in patients with acute hepatitis B.

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CLINICAL STUDIES

Efficacy of peginterferon-α-2b plus ribavirin in patients aged 65 years and older with chronic hepatitis C

Takashi Honda[1], Yoshiaki Katano[1], Junichi Shimizu[1], Yoji Ishizu[1], Masao Doizaki[1], Kazuhiko Hayashi[1], Masatoshi Ishigami[1], Akihiro Itoh[1], Yoshiki Hirooka[1], Isao Nakano[1], Fumihiro Urano[2], Kentaro Yoshioka[3], Hidenori Toyoda[4], Takashi Kumada[4] and Hidemi Goto[1]

1 Department of Gastroenterology, Nagoya University Graduate School of Medicine, Nagoya, Japan
2 Department of Gastroenterology, Toyohashi Municipal Hospital, Toyohashi, Japan
3 Division of Liver and Biliary Diseases, Department of Internal Medicine, Fujita Health University, Toyoake, Japan
4 Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan

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Correspondence
Yoshiaki Katano, Department of Gastroenterology, Nagoya University Graduate School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466-8550, Japan
Tel: +81 52 744 2169
Fax: +81 52 744 2178
e-mail: ykatano@med.nagoya-u.ac.jp

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Abstract

Objectives: The aim of this study was to evaluate the efficacy and indication of combination therapy with ribavirin plus peginterferon-α-2b in chronic hepatitis C virus (HCV) patients aged 65 years and older. *Methods:* Five hundred and ninety-one consecutive HCV patients were treated with combination therapy. These patients were divided into elder patients ($\geq$65 years) (n = 115) and younger patients (< 65 years) (n = 476). The clinical characteristics, sustained virological response (SVR) rates and discontinuation rates were compared between the two groups. *Results:* Compared with younger patients, baseline haemoglobin levels and baseline platelet counts were significantly lower ($P < 0.0001$, $P = 0.013$ respectively) and fibrosis was more advanced in elderly patients ($P = 0.0310$). Moreover, the SVR rate was significantly lower (37.4 vs. 51.5%; $P = 0.0067$) while the combination therapy discontinuation rate was significantly higher (32.2 vs. 17.0%; $P = 0.0003$) in elderly patients. A multivariate analysis revealed that HCV load and genotype were significantly associated with an SVR in elderly patients. An SVR was achieved in over 50% of elderly male patients with genotype 1 and HCV RNA concentrations under 2 000 000 IU/ml. In contrast, the SVR rate was under 30% in elderly male patients with genotype 1 and with HCV RNA concentrations over 2 000 000 IU/ml and in all elderly female patients with genotype 1. *Conclusions:* The SVR rate was lower in elderly patients than in younger patients. However, in elderly patients combination therapy was most beneficial for genotype 1 patients, male patients with HCV RNA concentrations < 2 000 000 IU/ml and patients with genotype 2.

Hepatitis C virus (HCV) infection is a widespread viral infection that often leads to chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). The need for chronic HCV therapies for elderly patients is increasing in Japan and is expected to rise in the US and other Western countries (1). Moreover, HCC has become a recent and growing problem in elderly patients with chronic hepatitis C.

Sustained virological responders who are negative for serum HCV RNA 6 months after interferon (IFN) treatment are reported to be likely to remain in virological and biochemical remission with histological improvement (2, 3). Moreover, IFN therapy reduces the risk of HCC among virological or biochemical responders (4–6). Ribavirin is now generally used in combination with IFN to treat chronic hepatitis C, and this combination therapy is reportedly more effective than IFN monotherapy, with a higher rate of HCV eradication (7–10).

It is important to determine whether elderly hepatitis C patients should be treated with IFN. Arase *et al.* (11) reported that HCV clearance after IFN therapy significantly reduced the risk of HCC and death in older hepatitis C patients. In addition, Veldt *et al.* (12) reported that a sustained virological response (SVR) to treatment is associated with improved clinical outcomes in the general population with chronic hepatitis C and advanced fibrosis.

Several studies have shown that IFN monotherapy has comparable efficacy in elderly and younger patients with chronic hepatitis C (13, 14). IFN and ribavirin combination therapy has greater efficacy than IFN monotherapy

(7, 9). However, elderly patients with genotype 1 and high HCV loads have a lower SVR rate than younger patients because of higher dose reduction rates and discontinuation rates because of ribavirin-related anaemia (15, 16). In a previous study, we examined patients with a similar background, except for age, and found that treating chronic hepatitis C with combination therapy was comparably effective between patients ≥ 60 years old and those < 60 years old, although the ribavirin discontinuation rate was higher among older patients (17). Similar results were obtained from chronic hepatitis patients treated with peginterferon and ribavirin; although the probability of a positive response to peginterferon-α plus ribavirin combination therapy was decreased for genotype 1- or 4-infected patients older than 40 years, patients older than 65 years had a response rate similar to those aged 40–64 years (18). There are few reports on the efficacy of ribavirin and peginterferon in the elderly patients with chronic hepatitis C. Moreover, no study has determined which patients will benefit from combination therapy among elderly patients with chronic hepatitis C. This study was designed to examine the background and treatment efficacy of peginterferon and ribavirin combination therapy according to gender in older patients with chronic hepatitis C and to identify which patients will achieve an SVR in this patient population.

Methods

Patients

This nonrandomized, prospective study was originally discussed in December 2004 by a committee composed of members from Nagoya University Hospital and 63 affiliated hospitals in Japan. Diagnostic criteria for chronic hepatitis C patients, peginterferon and ribavirin regimens and follow-up protocols were determined. Patients were divided by age into two groups: those aged ≥ 65 years and those aged < 65 years. Patients were compared with respect to background and treatment efficacy according to gender and tolerability of combination therapy with peginterferon and ribavirin. The study protocol was approved by the ethics committee of each hospital, and written informed consent was obtained from each patient before therapy.

Five hundred and ninety-one consecutive patients with chronic hepatitis C were treated with peginterferon and ribavirin combination therapy between December 2004 and February 2007 at 64 institutions: Nagoya University Hospital and affiliated hospitals. The indications for treatment were under 75 years old, positive for antibody to HCV and a serum HCV RNA level > 100 000 IU/ml by a quantitative PCR assay (Amplicor GT-HCV Monitor version 2.0; Roche Molecular Systems, Pleasanton, CA, USA) within 12 weeks preceding the treatment. In Japan, combination with peginterferon and ribavirin therapy for patients with an HCV RNA level > 100 000 IU/ml (high viral load in Japan) was approved for medical insurance coverage. Exclusion criteria included pretreatment haemoglobin (Hb) levels < 10 g/dl, positive for serum hepatitis B surface antigen, drug addiction, alcohol abuse, autoimmune hepatitis, primary biliary cirrhosis, human immunodeficiency virus, coexisting serious psychiatric or medical illness and pregnancy. To exclude any patient bias, only complete cohorts from each hospital were enrolled. HCV genotypes were determined by PCR with genotype-specific primers that were described previously by Ohno *et al.* (19). All genotyping was performed at one institution.

All patients were treated with 1.5 μg peginterferon-α-2b (Pegintron®; Schering-Plough K. K., Osaka, Japan) per kilogram of body weight subcutaneously once weekly for 24 weeks for genotype 2 patients and for 48 weeks for genotype 1 patients. When the virus was eradicated between 16 and 24 weeks from the beginning of treatment, the treatment duration was prolonged up to 72 weeks for genotype 1 patients. Treatment was discontinued when a patient's Hb concentration declined below 8.5 g/dl because of drug-induced haemolytic anaemia or when a patient's white blood cell count declined below 1000/mm^3, the neutrophil count declined below 500/mm^3 or the platelet count declined below 50 000/mm^3. Some patients discontinued treatment because the virus could not be eradicated after 24 weeks, as determined by the physician. We considered these cases to be discontinued. Oral ribavirin (Rebetol; Schering-Plough K. K.) was administered for the same duration as peginterferon at 600 mg/day for patients who weighed < 60 kg, 800 mg/day for those who weighed > 60 kg but < 80 kg and 1000 mg/day for those who weighed > 80 kg during the treatment period. The dose of ribavirin was reduced by 200 mg/day when the patient's Hb concentration declined below 10 g/dl because of drug-induced haemolytic anaemia. Ribavirin was discontinued when peginterferon therapy was discontinued. In Japan, peginterferon and ribavirin combination therapy was not approved for medical insurance coverage until November 2004.

Liver histology

Pretreatment liver biopsy specimens were analysed for fibrosis on a scale of F0–F4 (F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis) and for necroinflammatory activity on a scale of A0–A3 (A0, no histological activity; A1, mild activity; A2, moderate activity; and A3, severe activity) (20).

Assessment of efficacy

A virological response was assessed using a qualitative HCV RNA assay with a lower detection limit of 100 IU/ml (Amplicor HCV version 2.0; Roche Molecular Systems). According to the qualitative HCV RNA results, the responses were defined as follows: SVR (no HCV RNA

detected at the end of the 24-week follow-up period after completion of treatment), relapse (no HCV RNA at the end of treatment and reappearance of serum HCV RNA during the 24-week follow-up period) or nonresponse (persistent positive serum HCV RNA throughout treatment).

Comparison of characteristics and efficacy of treatment according to age

Patients were divided by age into two age groups: (1) ≥ 65 years old ($n = 115$) and (2) < 65 years old ($n = 476$). The following baseline parameters were compared between the two groups: gender ratio, age, body weight, body mass index (BMI), alanine aminotransferase (ALT) levels, γ-glutamyl transpeptidase (GGT), Hb levels, platelet counts, HCV genotype and viral load, histological activity and fibrosis. The SVR rate, rapid virological response (RVR) (HCV RNA negative by a qualitative assay at week 4) rate, early virological response (HCV RNA negative by a qualitative assay at week 12) rate and end of treatment virological response (ETR) rate were obtained by an intention-to-treat (ITT) analysis and per-protocol (PP) analysis, and the ribavirin or peginterferon reduction rate and combination therapy discontinuation rate were compared between the two age groups.

Comparison of treatment efficacy between patients who did and did not achieve a sustained virological response

To identify factors that predict an SVR among patients treated with combination therapy, we first determined the factors associated with an SVR in combination therapy with respect to the same factors above baseline parameters by a univariate analysis. Next, we identified the factors associated with an SVR in combination therapy, including gender, age, BMI, baseline serum ALT, GGT, Hb, platelet counts, genotype and HCV RNA, using a multivariate stepwise analysis with forward inclusion methods.

Comparison of treatment efficacy between older patients who did and did not achieved a sustained virological response

To identify elderly patients who may particularly benefit from combination therapy, we determined factors associated with an SVR using a univariate analysis of the same background factors as above. Then we determined factors associated with an SVR in elderly patients treated with combination therapy by a multivariate stepwise analysis with forward inclusion methods. In addition, we analysed the virological responses to combination therapy according to the age and gender of patients infected with each genotype because the age distribution of the treated patients differed according to gender.

Statistical analysis

Values are expressed as means ± standard deviation (SD). Between-group differences in the mean quantitative values were analysed using Student's *t*-test, and differences in nonparametrical data were analysed using the Mann–Whitney *U*-test. Differences in proportions were tested by a χ^2-test. The SVR rate between age generations in females was assessed using Fisher's exact test. Multiple logistic regression analysis was used to identify factors related to SVR. Statistical analyses were performed using SPSS software version 16.0 (SPSS Japan Inc., Tokyo, Japan) for multiple logistic regression analysis and SAS software (SAS Institute Inc., Cary, NC, USA) for another analysis. All *P* values were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 658 patients were screened, and 591 patients were enrolled in this study (Fig. 1). The patients included 327 men and 264 women aged 20–74 years (mean ± SD, 54.7 ± 11.6). Patients ≥ 65 years old comprised 19.5% of the patient population (115/591). The clinical characteristics of the two study groups are shown in Table 1. Body weight was significantly lower in patients aged ≥ 65 years than that in patients aged < 65 years ($P = 0.0006$). Hb levels and platelet counts were significantly lower in patients aged ≥ 65 years than those in patients aged < 65 years ($P < 0.0001$ and $P = 0.0013$ respectively). The fibrosis stage was more advanced in patients aged ≥ 65 years than that in patients aged < 65 years ($P = 0.0310$).

Response to therapy

The ribavirin dose reduction rate was significantly higher in patients aged ≥ 65 years than that in patients aged < 65 years ($P = 0.00013$) (Table 2), while the peginterferon dose reduction rate did not differ significantly between the two groups. The treatment discontinuation rate in patients aged ≥ 65 years was significantly higher than that in patients < 65 years ($P = 0.0003$). As a result, the SVR rate by ITT analysis in patients aged ≥ 65 years was significantly lower than that in patients aged < 65 years ($P = 0.0067$). However, the SVR and ETR rate by PP analysis were not significantly different between the two groups.

The factors associated with an SVR were determined by univariate analysis. The SVR rate was significantly higher in male patients than that in female patients ($P = 0.0153$) (Table 3). Age was significantly lower in patients who achieved an SVR than in patients who did not achieve an SVR ($P < 0.0001$). Hb levels and platelet counts were significantly higher in patients who achieved an SVR than those in patients who did not achieved an SVR ($P = 0.0202$ and $P = 0.0002$ respectively). The HCV load in patients who achieved an SVR was significantly lower than that in patients who did not achieved an SVR

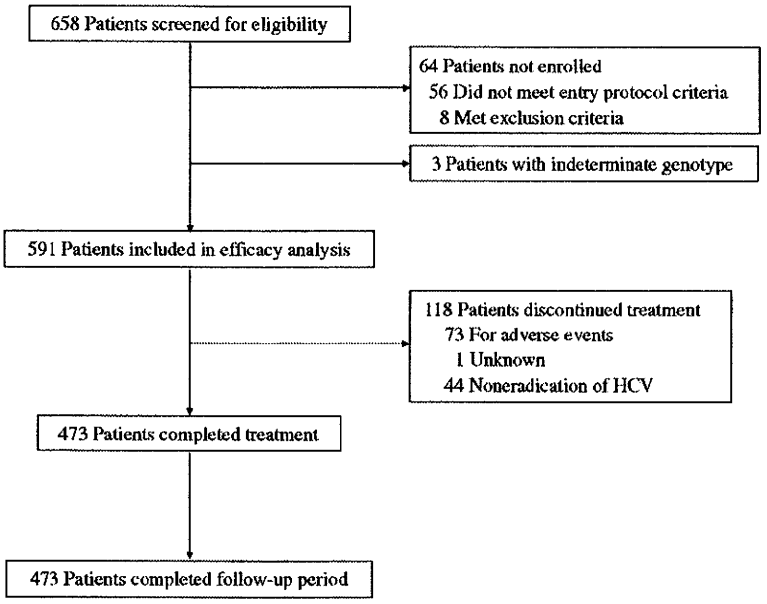

Fig. 1. Flow chart for patient selection.

Table 1. Baseline clinical characteristics of patients treated with combination therapy

	Total patients ($n=591$)	Patients aged < 65 years ($n=476$)	Patients aged ≥ 65 years ($n=115$)	*P* value
Sex ratio (male/female)	327/264	270/206	57/58	0.1659
Age (years)	54.7 ± 11.6	51.5 ± 10.6	67.9 ± 2.2	< 0.0001
Body weight (kg)	60.1 ± 11.3	60.9 ± 11.4	56.7 ± 10.1	0.0006
Body mass index	22.9 ± 3.2	22.9 ± 3.2	22.9 ± 3.2	0.9221
Baseline serum ALT (IU/L)	64.8 ± 57.3	66.5 ± 60.6	57.7 ± 40.4	0.1425
GGT (IU/L)	57.8 ± 76.7	58.9 ± 78.9	53.3 ± 67.3	0.4880
Haemoglobin (g/dl)	14.1 ± 1.3	14.2 ± 1.4	13.7 ± 1.2	< 0.0001
Platelets (× 10^4/μl)	17.7 ± 5.7	18.0 ± 5.9	16.1 ± 4.3	0.0013
Genotype (1/2)	467/124	374/102	93/22	0.5870
HCV RNA (kIU/ml)	1863.3 ± 1456.3	1896.4 ± 1454.9	1726.2 ± 1460.5	0.2611
Activity (A0/A1/A2/A3)	16/255/141/19	13/202/115/13	3/53/26/6	0.6053
Fibrosis (F0/F1/F2/F3/F4)	37/228/107/56/5	31/191/83/37/3	6/37/24/19/2	0.0310

ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; HCV RNA, hepatitis C virus RNA; kIU, kilo international units.

($P=0.0132$). The rate of genotype 2 patients who achieved an SVR was significantly higher than that in patients who did not achieve an SVR ($P < 0.0001$). The fibrosis stage was more advanced in patients who did not achieve an SVR than that in those who did achieve an SVR ($P=0.0186$).

The factors associated with an SVR in combination therapy were determined by multivariate analysis (Table 4). Age [$P < 0.0001$, odds ratio 0.959 (0.942–0.975)] and genotype [$P < 0.0001$, odds ratio 0.415 (0.255–0.676)] were significantly associated with an SVR. Including the RVR (ITT) factor after starting treatment, the factors associated with an SVR in combination therapy were determined using a multivariate analysis (Table 5). Age [$P < 0.0001$, odds ratio 0.961 (0.944–0.978)] and RVR [$P < 0.0001$, odds ratio 8.168 (4.511–14.789)] were significantly associated with an SVR.

Then, we separately analysed male and female patients in different age groups. The virological responses to combination therapy according to the gender of genotype 1 patients are shown by age groups in Figure 2. In both males and females, the SVR rate decreased with age, and the SVR rates of patients < 40 years old were over 50%. In patients aged ≥ 65 years, the SVR rate of female patients was lower than that in patients aged < 65 years and was lower than that of male patients aged ≥ 65 years [20.8% (10/48) vs. 40.5% (64/158) and 20.8% (10/48) vs. 42.2% (19/45); $P=0.0261$ respectively].

Table 2. Efficacy of combination therapy

	Total patients ($n=591$)	Patients aged < 65 years ($n=476$)	Patients aged ≥65 years ($n=115$)	*P* value
SVR rate (intention-to-treat)	48.7 (288/591)	51.5 (245/476)	37.4 (43/115)	0.0067
SVR rate (per-protocol)	59.2 (280/473)	60.3 (238/395)	53.8 (42/78)	0.2927
RVR rate (intention-to-treat)	20.0 (118/591)	21.2 (101/476)	14.8 (17/115)	0.1213
RVR rate (per-protocol)	22.0 (104/473)	22.8 (90/395)	17.9 (14/78)	0.3460
EVR rate (intention-to-treat)	62.6 (370/591)	64.5 (307/476)	54.8 (63/115)	0.0534
EVR rate (per-protocol)	71.0 (336/473)	71.1 (281/395)	70.5 (55/78)	0.9113
ETR rate (intention-to-treat)	81.0 (479/591)	83.2 (396/476)	72.2 (83/115)	0.0068
ETR rate (per-protocol)	92.8 (439/473)	92.9 (367/395)	92.3 (72/78)	0.8504
Ribavirin dose reduction rate	43.1 (255/591)	39.9 (190/476)	56.5 (65/115)	0.0013
PEGIFN dose reduction rate	34.3 (203/591)	33.2 (158/476)	39.1 (45/115)	0.2289
Combination therapy discontinuation rate	20.0 (118/591)	17.0 (81/476)	32.2 (37/115)	0.0003
Combination therapy discontinuation rate*	12.5 (74/591)	9.9 (47/476)	23.5 (27/115)	< 0.0001

*Except genotype 1 patients which therapy was discontinued because the virus could not be eradicated after 24 weeks.
ETR, end of treatment virological response; EVR, early virological response; PEGIFN, peginterferon; RVR, rapid virological response; SVR, sustained virological response.

Table 3. Factors associated with a sustained virological response in combination therapy by a univariate analysis

	Total patients ($n=591$)	Patients who achieved an SVR ($n=288$)	Patients who did not achieved an SVR ($n=303$)	*P* value
Sex ratio (male/female)	327/264	171/114	153/150	0.0153
Age (years)	54.7 ± 11.6	51.9 ± 12.6	57.3 ± 9.8	< 0.0001
Body weight (kg)	60.1 ± 11.3	60.7 ± 10.8	59.6 ± 11.8	0.2661
Body mass index	22.9 ± 3.2	23.0 ± 2.9	22.9 ± 3.5	0.8785
Baseline serum ALT (IU/L)	64.8 ± 57.3	65.8 ± 63.8	63.8 ± 50.5	0.6758
GGT (IU/L)	57.8 ± 76.7	54.7 ± 91.0	60.7 ± 60.2	0.3425
Haemoglobin (g/dl)	14.1 ± 1.3	14.2 ± 1.4	14.0 ± 1.3	0.0202
Platelets ($\times 10^4/\mu l$)	17.7 ± 5.7	18.6 ± 6.1	16.8 ± 5.1	0.0002
Genotype (1/2)	467/124	204/84	263/40	< 0.0001
HCV RNA (kIU/ml)	1863.3 ± 1456.3	1711.2 ± 1415.4	2007.8 ± 1482.0	0.0132
Activity (A0/A1/A2/A3)	16/255/141/19	7/121/70/9	9/134/71/10	0.9596
Fibrosis (F0/F1/F2/F3/F4)	37/228/107/56/5	18/122/46/20/0	19/106/61/36/5	0.0186

ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; HCV RNA, hepatitis C virus RNA; kIU, kilo international units.

Table 4. Multivariate analysis of factors associated with a sustained virological response in combination therapy

Variable		Odds ratio (95% CI)	*P* value
Age		0.959 (0.942–0.975)	< 0.0001
Genotype	1 vs. 2	0.415 (0.255–0.676)	< 0.0001

CI, confidence interval.

Table 5. Multivariate analysis of factors (including treatment response) associated with a sustained virological response in combination therapy

Variable		Odds ratio (95% CI)	*P* value
Age		0.961 (0.944–0.978)	< 0.0001
RVR	RVR vs. nonRVR	8.168 (4.511–14.789)	< 0.0001

CI, confidence interval; RVR, rapid virological response.

Virological responses to combination therapy according to the gender of genotype 2 patients are shown by age groups in Figure 3.

The SVR rate was similar for all age groups among male patients. In both male and female patients < 40 years old, the SVR rate was over 75%. In both male and female patients over 40 years old, the SVR rate was approximately 60%.

Response to therapy in older patients

In patients aged ≥65 years, the factors associated with an SVR were determined by univariate analysis (Table 6). The SVR rate of male patients was significantly higher than that of female patients ($P=0.0284$). The ratio of genotype 1 in patients who achieved an SVR was significantly lower than that in patients who did not

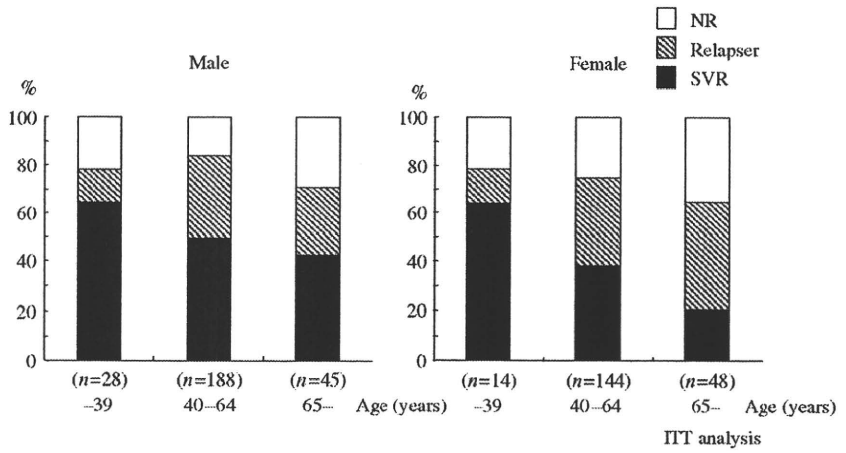

Fig. 2. A virological response to combination therapy according to the age and gender of patients with genotype 1. ITT, intention-to-treat; NR, nonresponder; SVR, sustained virological response.

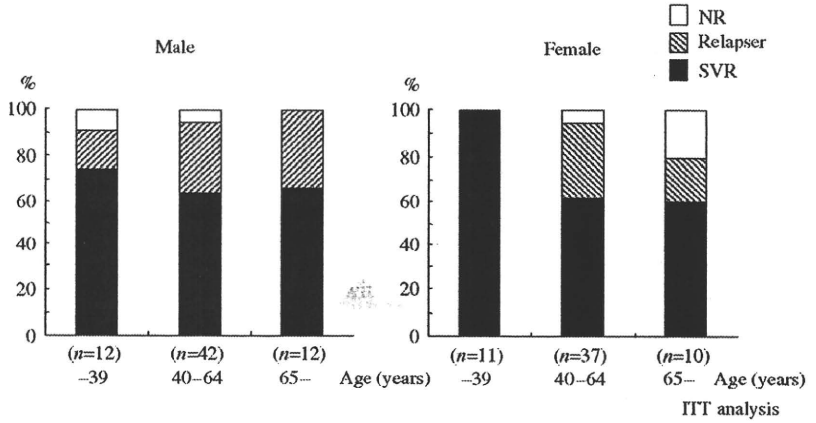

Fig. 3. A virological response to combination therapy according to the age and gender of patients with genotype 2. ITT, intention-to-treat; NR, nonresponder; SVR, sustained virological response.

Table 6. Univariate analysis of factors associated with sustained virological response in patients aged ≥65 years treated with combination therapy

	Total patients ($n=115$)	Patients who achieved a SVR ($n=43$)	Patients who did not achieved a SVR ($n=72$)	*P* value
Sex ratio (male/female)	57/58	27/16	30/42	0.0284
Age (years)	67.9 ± 2.2	67.9 ± 2.3	67.8 ± 2.1	0.7666
Body weight (kg)	56.7 ± 10.1	56.9 ± 7.1	56.5 ± 11.4	0.8417
Body mass index	22.9 ± 3.2	22.8 ± 1.9	23.0 ± 3.7	0.6980
Baseline serum ALT (IU/L)	57.7 ± 40.4	57.2 ± 41.3	58.0 ± 40.2	0.9178
GGT (IU/L)	53.3 ± 67.3	61.2 ± 98.3	48.8 ± 40.4	0.3471
Haemoglobin (g/dl)	13.7 ± 1.2	13.8 ± 1.2	13.6 ± 1.3	0.3341
Platelets ($\times 10^4/\mu l$)	16.1 ± 4.3	16.3 ± 4.7	16.0 ± 4.1	0.7412
Genotype (1/2)	93/22	29/14	64/8	0.0047
HCV RNA (kIU/ml)	1726.2 ± 1460.5	1383.6 ± 1247.0	1930.9 ± 1546.4	0.0514
Activity (A0/A1/A2/A3)	3/53/26/6	1/17/10/4	2/36/16/2	0.4132
Fibrosis (F0/F1/F2/F3/F4)	6/37/24/19/2	0/16/9/7/0	6/21/15/12/2	0.2538

ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; HCV RNA, hepatitis C virus RNA; kIU, kilo international units; SVR, sustained virological response.

achieve an SVR ($P = 0.0047$). The HCV load tended to be lower in patients who achieved an SVR than that in patients who did not achieve a SVR ($P = 0.0514$).

In patients aged ≥65 years, the factors associated with an SVR with combination therapy were determined using multivariate analysis (Table 7). Viral load [$P = 0.015$, odds ratio 1.000 (0.999–1.000)] and genotype [$P = 0.022$, odds ratio 0.268 (0.087–0.830)] were significantly associated with an SVR. Gender [$P = 0.052$, odds ratio 0.410 (0.166–1.009)] tended to be associated with an SVR.

To identify patients aged ≥65 years with genotype 1 (hard-to-treat population) who may benefit from combination therapy, we examined the efficacy of combination therapy according to viral load and gender (Fig. 4). Even among older male patients with high viral loads, patients with viral loads < 2 000 000 IU/ml had a significantly higher SVR than patients with viral loads over 2 000 000 IU/ml [56.7% (17/30) vs. 13.3% (2/15)] ($P = 0.0094$). In contrast, there was no significant difference in the SVR rate between older female patients with viral loads < 2 000 000 IU/ml and those with viral loads over 2 000 000 IU/ml.

To evaluate the ribavirin dose during the first quarter (12 weeks) in each group of genotype 1 patients at two institutions, we calculated the percent intake of the expected dose during the first quarter. The percentage of patients who achieved a drug intake rate over 80% during the first quarter was significantly lower in elderly patients than in younger patients (75.0 vs. 88.4%; $P = 0.0442$). Similarly, the patients who achieved an SVR were more likely to have a drug intake rate over 80% than patients who did not achieve an SVR (91.7 vs. 80.7%; $P = 0.0464$).

Table 7. Multivariate analysis of factors associated with a sustained virological response in patients aged ≥65 years treated with combination therapy

Variable		Odds ratio (95% CI)	*P* value
HCV RNA (kIU/ml)		1.000 (0.999–1.000)	0.015
Genotype	1 vs. 2	0.268 (0.087–0.830)	0.022
Gender	Female vs. male	0.410 (0.166–1.009)	0.052

HCV RNA, hepatitis C virus RNA; kIU, kilo international units; SVR, sustained virological response.

Adverse events

The combination therapy discontinuation rate of patients aged ≥65 years was significantly higher than that of patients aged < 65 years ($P = 0.0003$) (Table 2). Even when excluding genotype 1 cases in which therapy was discontinued because the virus could not be eradicated after 24 weeks, the combination therapy discontinuation rate of patients aged ≥65 years was significantly higher than that of patients aged < 65 years ($P < 0.0001$). Ribavirin discontinuation was higher in older patients ($P = 0.0013$). The reasons for discontinuing combination therapy and the times when therapy was discontinued are shown in Table 8. One case with a serious adverse effect occurred in each group: insulin-dependent diabetes mellitus in the younger group and bleeding from duodenal varices in the older group. The discontinuation rate because of general fatigue or anaemia was higher in older patients than that in younger patients [5.22% (6/115) vs. 1.90% (9/476) ($P = 0.0418$) and 5.22% (6/115) vs. 0.63% (3/476) ($P = 0.0024$) respectively].

Discussion

It is important to eradicate HCV by IFN to reduce the risk of HCC (4, 5). In addition, IFN reportedly reduces liver-related mortality in chronic hepatitis C patients over age 60 years old (11, 21, 22). However, these findings are based on studies of IFN monotherapy. The present study examined the effect of a combination of ribavirin and peginterferon. Ribavirin has been used in combination with IFN or peginterferon to treat chronic hepatitis C, and this combination therapy has been reported to be more effective than IFN monotherapy in eradicating

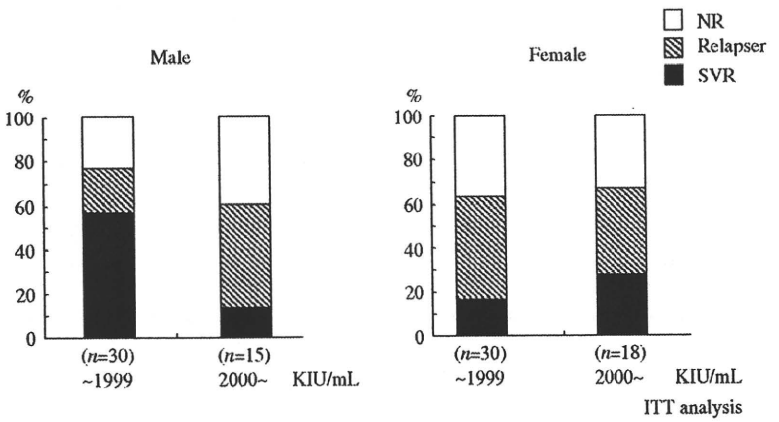

Fig. 4. A virological response to combination therapy according to virus load and gender of older patients with genotype 1. ITT, intention-to-treat; kIU, kilo international units; NR, nonresponder; SVR, sustained virological response.

Table 8. Reasons for discontinuing combination therapy

Reason	Number	Weeks after starting treatment	Reason	Number	Weeks after starting treatment
Patients aged < 65 years (n = 476)			*Patients aged ≥ 65 years (n = 115)*		
Fatigue	9	4, 8, 8, 10, 13, 20, 25, 33	Fatigue*	6	1, 4, 6, 8, 19, 32
Depression	7	1, 2, 4, 8, 13, 15, 18	Anaemia*	6	3, 8, 12, 12, 13, 15
Self-discontinuation	6	8, 16, 23, 24, 25, 28	Rash	3	1, 4, 9
Headache	3	2, 36, 37	Depression	2	2, 9
Anaemia	3	4, 11, 24	Jaundice	1	1
Rash	2	18, 25	Fiver	1	7
Hepatocellular carcinoma	2	19, 43	**Bleeding from duodenal varies‡**	**1**	**8**
Bronchitis	1	2	Anorexia	1	10
Alopecia	1	13	Hyperthyroidism	1	15
Progression of diabetes	1	14	Cholecystitis	1	16
Peritonitis due to appendicitis	1	16	Symptoms of Parkinson's disease	1	16
Fundal hemorrhage	1	17	Suspicion of interstitial pneumonia	1	20
Pneumonia	1	18	Gastric cancer	1	21
Body weight loss	1	22	Hepatocellular carcinoma	1	21
Vertigo	1	25			
Elevation of TSH	1	25			
Unknown	1	25			
Lack of funds	1	27			
Hypothyroidism	1	28			
Gastric cancer	1	38			
Insulin-dependent diabetes mellitus‡	**1**	**44**			
Reappearance of pancreatitis	1	46			
Noneradication of HCV†	34		Noneradication of HCV†	10	

*The ratio of discontinuation was significantly different between the two groups $P < 0.05$.
†Discontinued because the virus could not be eradicated after 24 weeks.
‡Serious adverse effects are shown in bold.

HCV (7–10). However, ribavirin and IFN or peginterferon in combination produce a common adverse effect, i.e. Hb levels decrease in 20–36% of treated patients with chronic hepatitis C, necessitating dose reduction or discontinuation (8, 10, 23, 24). Among elderly patients treated with combination therapy, ribavirin dose reduction is often required, resulting in a reduced SVR in older patients (15, 16). In this study, ribavirin dose reduction was higher in elderly patients than that in younger patients.

Previous studies have reported that there is no significant difference in the efficacy of IFN monotherapy between older and younger patients after normalizing for difference in background clinical characteristics, suggesting that age does not influence the outcome of IFN monotherapy (13, 14).

Adding ribavirin to IFN improves the treatment efficacy. However, ribavirin reduces Hb levels, causing greater dose reductions. Elderly patients with genotype 1 and high HCV loads have a lower SVR rate than younger patients because of a higher ribavirin dose reduction rate and discontinuation rate because of ribavirin-related anaemia (15, 16). We examined chronic hepatitis C patients with a similar background, except for age, and found that combination therapy was comparably effective between patients aged ≥60 years and those aged < 60 years, although the ribavirin discontinuation rate was higher among older patients (17). Similar results were obtained in the chronic hepatitis C patients treated with peginterferon and ribavirin, and positive responses to combination treatment were decreased for genotype 1- or 4-infected patients older than 40 years, but comparable between patients older than 65 and patients aged 40–64 years (18).

However, the background, efficacy and tolerability of peginterferon and ribavirin combination therapy in elderly patients according to gender have not been fully elucidated. Moreover, there are no data identifying which patients will achieve an SVR among older patients. Our previous report examined a 24-week regimen of ribavirin plus interferon therapy and defined advanced age as over 60 years (17). However, currently, the most common treatment protocol is prolonged ribavirin plus peginterferon-α treatment. Moreover, the patient age distribution has shifted to a more advanced age. Therefore, we need to re-evaluate an additional protocol including peginterferon and define advanced age as 65 years. We conducted a multi-institution study to evaluate the efficacy and tolerability of ribavirin plus peginterferon-α in older patients with chronic hepatitis C.

An ITT analysis indicated that the SVR rate in elderly patients was lower than that in younger patients, while a PP analysis showed that the SVR rate in elderly patients was not statistically different from that of younger

patients. These results indicated that when treatment is not discontinued, the SVR rate of elderly patients will be high.

Multivariate analysis showed that baseline age and genotype are factors significantly associated with an SVR. Many studies have shown that baseline viral load and genotype are factors significantly associated with an SVR (8, 24). Age was associated with an SVR and the SVR rate of patients aged ≥65 years was lower than that of patients aged < 65 years (37.4 vs. 51.5%; $P=0.0067$).

Because the SVR differs according to genotype, we classified patients by genotype and compared the SVR rate for both male and female patients. In both male and female patients with genotype 1, the SVR rate decreased with age, and the SVR rate of both patients < 40 years was over 60%. These results were similar to previous studies where the SVR rate of patients < 40 years old was higher than that of another generation (17, 18, 24, 25). In patients ≥65 years old, the SVR rate of female patients was significantly lower than that of male patients [in patients with both genotype 1 and 2, 27.6% (16/58) vs. 47.4% (27/57); $P=0.0284$; in patients with genotype 1, 20.8% (10/48) vs. 42.2% (19/45); $P=0.0261$]. The result that female patients are less likely to achieve an SVR than male patients differs from that of a previous report (25). However, our results are consistent with Sezaki and colleagues, who reported that females have a poorer response to peginterferon and ribavirin combination therapy than males among patients with hepatitis C aged ≥50 years. In the older population, the gender associated with an SVR changes from male to female (26). In both male and female patients with genotype 2, the SVR of all generations was over 60%. A study by Antonucci *et al.* (18)and our previous report suggest that genotype 2 patients have a higher SVR rate, which is age-independent.

We cannot exclude the bias that better candidates were more likely to be selected among older patients than younger patients in the outpatient department. However, regardless of potential bias, elderly patients had low body weight, low Hb levels and an advanced fibrosis stage. Regarding fibrosis, elderly patients are more likely to have a long disease duration as it was reported previously that fibrosis progression was mainly dependent on age and the duration of infection (27). In this study, ITT and PP analyses indicated that the SVR rate did not differ between younger and older patients with F2–F4 fibrosis who needed treatment to prevent liver-related deaths (data not shown).

Several reports suggested that the efficacy of peginterferon and ribavirin combination therapy is lower in elderly patients with genotype 1 than in younger patients, but there are no reports establishing which elderly patients will benefit from this combination therapy. To identify genotype 1 patients ≥65 years old (hard-to-treat population) who will particularly benefit from combination therapy, we examined the efficacy of combination therapy according to viral load and gender. In older male patients with genotype 1 and HCV RNA concentrations < 2 000 000 IU/ml, the SVR rate was over 50%. Based on these results, combination therapy should be considered for male patients with genotype 1 and with HCV RNA concentrations < 2 000 000 IU/ml. Even if the treatment schedules differ between western countries and Japan, age will have to be considered, and viral load will be an important issue when treating elderly patients.

In conclusion, elderly patients in Japan who received combination therapy with peginterferon and ribavirin had a low body weight, low Hb levels and advanced fibrosis. Elderly patients had higher treatment discontinuation rates and lower SVR rates than younger patients. However, an SVR was achieved in over 50% of elderly patients with genotype 2 and in male patients with genotype 1 and HCV RNA concentrations < 2 000 000 IU/ml.

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Appendix 1

The following institutions participated in this study:

- Aihoku Hospital
- Aichi Cancer Center
- Aichi Cancer Center Aichi Hospital
- Aichi Saiseikai Hospital
- Aichi Sannomaru Hospital
- Atsumi Hospital
- Anjo Kosei Hospital
- Ichinomiya Municipal Hospital
- Ichinomiya Municipal Hospital Imaise Branch
- Inazawa City Hospital
- Ogaki Municipal Hospital
- Okazaki City Hospital
- Kainan Hospital
- Kakegawa City General Hospital
- Kamo Hospital
- Kariya Toyota General Hospital
- Gifu Social Insurance Hospital
- KumiaiKosei Hospital
- Aichi Cardiovascular and Respiratory Center
- Showa Hospital
- Tosei General Hospital
- Komaki City Hospital
- Komaki Daiichi Hospital
- Sakashita Hospital
- Saishukan Hospital
- Shizuoka Kosei Hospital
- Shizuoka Saiseikai General Hospital
- Yokkaichi Municipal Hospital
- Holy Spirit Hospital
- Kamiiida daiichi General Hospital
- Daido Hospital
- Chita City Hospital
- Chubu Rosai Hospital
- National Center for Geriatrics and Gerontology
- Tsushima City Hospital
- Tokai Memorial Hospital
- Tokai Sangyo Central Hospital
- Tokai Municipal Hospital
- Tokai Central Hospital
- Tokai Hospital
- Tohno Kousei Hospital

- Toki General Hospital
- Tokoname Municipal Hospital
- Toyota Memorial Hospital
- Toyohashi Medical Center
- Toyohashi Municipal Hospital
- Nakatsugawa Municipal General Hospital
- Nagoya Medical Center
- Nagoya Ekisaikai Hospital
- Nagoya Memorial Hospital
- Nagoya Kyouritu Hospital
- Japanese Red Cross Nagoya First Hospital
- Nishio Municipal Hospital
- Handa City Hospital
- Fukuroi Municipal Hospital
- Fujita Health University Hospital
- Brother Hospital
- Hekinan Municipal Hospital
- Mitsubishi Nagoya Hospital
- Miyoshi Municipal Hospital
- Meijo Hospital
- Meitetsu Hospital
- Yachiyo Hospital
- Yamashita Hospital

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＜速　報＞

生存分析からみた進行肝細胞癌に対するソラフェニブ投与例の検討

多田　俊史*　熊田　卓　桐山　勢生　豊田　秀徳

緒言：ソラフェニブはSHARP試験[1]やAsia-Pacific試験[2]で延命効果が報告され，進行肝細胞癌に対する治療として重要な役割を担っている．

今回，ソラフェニブ投与例の生存分析を行い，投与開始時のどのような因子が予後に関係したかを検討した．

対象と方法：対象は2009年6月から2010年5月の間に当院でソラフェニブを開始した26例である．Child-Pugh（C-P）分類はA/Bが19/7例で，門脈侵襲はVp0/1/2/3/4が13/0/5/3/5例，肝外転移は肺/リンパ節/骨/副腎/脾臓が8/4/2/1/1例（重複あり）であった．初回治療は肝切除/TACE/RFA/TACE＋RFA/PEIT/肝動注化学療法が13/7/1/1/1/1例で，前治療なしが2例であった．ソラフェニブ投与前の治療はTACE/肝動注化学療法/肝切除/PEITが18/3/2/1例であった．なおTACE18例のうちVp2以下/Vp3以上が16/2例で，Vp2以下16例のうち肝外転移なし/ありが7/9例であった．Vp2以下かつ肝外転移なしの症例でTACEが不可能と判断された理由は，乏血性でTACEの効果が低いと考えられた，APシャントや肝外からの側副血行路の発達，Vp2であるが主治医の判断によるもの，が3例，2例，2例であった．観察期間中央値は4カ月（1-12カ月）で，観察期間中に死亡は13例であった．投与開始後，死亡までの期間は1カ月未満，1～3カ月未満，3～6カ月未満，6カ月以上がC-P分類A/B別に0/0例，3/4例，2/0例，2/2例であった．これらの患者の初回治療，C-P分類，肝外転移，門脈侵襲，AFP，AFP-L3分画，PIVKA-IIに関する生存分析を行った．

結果：Kaplan-Meier法による初回治療肝切除(n＝13)/非切除（n＝13），C-P分類A（n＝19）/B（n＝7），肝外転移なし（n＝13）/あり（n＝13），門脈侵襲Vp2以下（n＝18）/Vp3以上（n＝8），AFP 200 ng/ml未満（n＝12）/200以上（n＝14），AFP-L3分画陰性（10%未満）（n＝9）/陽性（n＝17），PIVKA-II 40 mAU/ml未満（n＝3）/40以上（n＝23）の5カ月生存率は76.9%/36.9%，61.6%/42.9%，46.2%/67.3%，72.2%/25.0%，83.3%/34.3%，88.9%/33.2%，100%/50.9%であった．

単変量解析では門脈侵襲がハザード比：3.672，95%信頼区間：1.272-10.604，p＝0.016と有意差が認められた（Table 1）．

さらに多変量解析（変数増加法）では，門脈侵襲がハザード比：4.631，95%信頼区間：1.541-13.921，p＝

Table 1　Univariate analysis for patient survival (Cox-proportional hazard model)

	Hazard ratio	95% C.I.	p value
Initial treatment (surgery/non-surgery)	2.594	0.811-7.638	0.084
Child-Pugh class (A/B)	1.922	0.674-5.480	0.222
Extrahepatic metastasis (absent/present)	0.738	0.266-2.048	0.738
Portal vein invasion (≦ Vp2/≧ Vp3)	3.672	1.272-10.604	0.016
AFP (< 200 ng/ml/≧ 200 ng/ml)	2.514	0.845-7.477	0.097
AFP-L3 (< 10%/≧ 10%)	3.444	0.958-12.379	0.058
PIVKA-II (< 40 mAU/ml/≧ 40 mAU/ml)	32.781	0.123-8731.448	0.221

C.I., confidence interval; AFP, alpha-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of AFP; PIVKA-II, protein induced by vitamin K absence or antagonist-II.

大垣市民病院消化器科
*Corresponding author: tadat@msd.biglobe.ne.jp
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0.006, AFPがハザード比：3.261, 95％信頼区間：1.049-10.140, $p=0.041$がそれぞれ生存に関与する因子として選択された.

考察：今回の検討では，単および多変量解析で門脈侵襲がVp3以上の症例で有意に生存率が低く，さらに生存に関与する因子として選択された．本邦では門脈侵襲があってもVp1，Vp2の場合はC-P分類もしくは肝障害度を考慮しつつ肝切除やさらにはTACEも広く行われている．肝外転移の出現や，腫瘍因子もしくは技術的な問題でTACEが不可能な場合であっても，Vp2以下であればソラフェニブの治療効果が期待できることが示唆された.

索引用語：肝細胞癌，ソラフェニブ，門脈侵襲

文献：1) Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in Advanced Hepatocellular Carcinoma. N Engl J Med 2008; 359: 378—390　2) Cheng A, Kang Y, Chen Z, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled traial. Lancet Oncol 2009; 10: 25—34

英文要旨

Survival analysis of Sorafenib in patients with advanced hepatocellular carcinoma

Toshifumi Tada*, Takashi Kumada, Seiki Kiriyama, Hidenori Toyoda

We investigated the pretreatment factors that were associated with the outcome of patients with hepatocellular carcinoma (HCC) who received the administration of sorafenib. Survival rates were compared according to the initial treatment for HCC, Child-Pugh class, extrahepatic metastasis, portal vein invasion, AFP, AFP-L3, and PIVKA-II, in 26 HCC patients who were administered sorafenib. Univariate analysis revealed the state of portal vein invasion (≤Vp2 vs. ≥Vp3) as a factor that was associated patient survival ($p=0.016$). By multivariate analysis, the state of portal vein invasion (hazard ratio, 4.631; 95％ confidence interval, 1.541-13.921; $p=0.006$) was selected as factors that affected patient survival. The present result shows that portal vein invasion of ≥Vp3 indicated poor outcome in patients with HCC treated by sorafenib.

Key words: hepatocellular carcinoma, sorafenib, portal vein invasion

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Department of Gastroenterology, Ogaki Municipal Hospital, Gifu, Japan

*Corresponding author: tadat@msd.biglobe.ne.jp

□□特集□□

肝臓のEOB・プリモビスト造影MRI －本検査を有効に活用するための理解すべき課題－

4. 肝細胞癌

4-3. 肝細胞造影相でhypointensityを呈する乏血性結節(非濃染結節)のリスクについて

小川　定信[1)]，川地　俊明[1)]，船坂　佳正[1)]，石川　照芳[1)]，
熊田　卓[2)]，豊田　秀徳[2)]，曽根　康博[3)]

大垣市民病院　診療検査科[1)]，同　消化器科[2)]，同　放射線科[3)]

The Risk of Hypovascular Nodules Showing Hypointensity during the Hepatocyte Enhanced Phase

Sadanobu Ogawa[1)], Toshiaki Kawachi[1)], Yoshimasa Funasaka[1)], Teruyoshi Ishikawa[1)], Takashi Kumada[2)], Hidenori Toyoda [2)], Yasuhiro Sone[3)]

1) Department of Clinical Research, Ogaki Municipal Hospital
2) Department of Digestive Organs, Ogaki Municipal Hospital
3) Department of Radiology, Ogaki Municipal Hospital

NICHIDOKU-IHO
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Summary

The diagnosis of liver nodules by MRI has been advanced greatly by the development of Gd-EOB-DTPA. In addition to tumor vascularity, it has become possible to detect lesions by observing differences in contrast medium concentration between the nodule and liver parenchyma during the hepatocyte enhanced phase. However, visualizing hypovascular nodules may be a problem. Observing those nodules carefully, we classified nodules into hypervascular or not, and compared findings based on several items.

As a result, none of the hypovascular nodules showed hypervascular change during the observation period, but the rate of hypervascular change increased at a nodule size of 15mm. We should carefully observe these nodules. Moreover, EOB-MRI is regularly performed in patients with chronic liver disease, and it is necessary to understand the risk in each individual patient.

はじめに

従来、肝臓におけるMRI検査は、各種シークエンスの信号強度と造影剤(gadolinium diethylenetriamine pentaacetic acid：Gd-DTPA)を用いたdynamic studyによる血流評価で病変の存在診断や質的診断がなされていた。ハード面の改良・発達により撮像時間の短縮や高磁場化によるS/N比の改善などMRI検査そのものの質的向上は図られてきたが、診断能に大きな変化は認められなかった。しかし、2008年1月に、バイエル薬品株式会社よりgadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)が販売され、MRIにおける肝腫瘤性病変の診断は大きく進歩した。本剤はGd-DTPAを基本骨格として、側鎖には脂溶性を示すエトキシベンジル基(ethoxybenzyl：EOB)が導入されている。MRI検査で一般に用いられているGd-DTPAは水溶性を示すため、臓器や組織に特異的に分布することはないが、本剤は脂溶性側鎖であるEOBの導入により投与された造影剤の一部が肝細胞に取り込まれる。このため肝細胞に造影剤の取り込まれた時相(肝細胞造影相)では、肝細胞機能に異常を示した病巣部と正常肝実質との間に造影剤濃度の分布差を生じることとなり、病巣を検出することが可能となる。また血管内投与直後には、Gd-DTPAと同様に非特異的に血管および細胞間隙に分布するため、dynamic studyによる腫瘍の血流動態を評価することができるため、血流評価に加えて機能面から