species production and the overexpression of MDR1mRNA in HepG2 cells. These results suggested that UDCA and CDCA inhibited the up-regulation of P-gp by eliminating DOX-induced reactive oxygen species and down-regulating cell signaling mediated by reactive oxygen species. The promoter region in the MDR1 gene has been shown to contain several elements responsive to NF-κB, SP-1, and PXR (Labialle et al., 2002; Bentires-Alj et al., 2003; Cornwell and Smith, 1993; Hu et al., 2000; Kurose et al., 2005). Although their exact role has not yet been clarified, reactive oxygen species has been shown to activate NF-κB signaling (Sen and Packer, 1996). On the other hand, it has been indicated that cadmium induced up-regulation of the MDR1 gene by activating NF-κB, and that this up-regulation was inhibited by antioxidants, NAC and pyrrolidine dithiocarbamate, suggesting that up-regulation of the MDR1 gene by cadmium may be mediated by reactive oxygen species and NF-κB signaling (Thévenod et al., 2000). These findings suggest that the DOX-induced overexpression of MDR1 mRNA and P-gp may be dependent on DOX-induced reactive oxygen species production and subsequent activation of NF-κB cell signaling and that UDCA inhibits these DOX-induced effects.

In this study, UDCA and CDCA inhibited the DOX-induced up-regulation of P-gp and UDCA reversed the decreased uptake of Rho123. Thus, UDCA exerted a marked effect on membrane transport function in DOX-treated HepG2 cells. CDCA has been shown to induce reactive oxygen species production and apoptosis in cells (Chu et al., 2003; Barrasa et al., 2011; Rosignoli et al., 2008). Indeed, our preliminary data indicated that 100 μM of CDCA slightly increased reactive oxygen species levels after incubation for 24 h, while UDCA did not up-regulate reactive oxygen species levels in HepG2 cells (data not shown). UDCA is well-known to be relatively hydrophilic and less cytotoxic than CDCA and the secondary bile acids. Considering these findings, the pre- and co-administration of UDCA should have the beneficial effect of regulating P-gp overexpression and preventing the acquisition of antitumor multidrug resistance in hepatocellular carcinoma treated with DOX or other anthracycline antitumor agents.

5. Conclusions

UDCA inhibited the DOX-induced overexpression of P-gp and restored the DOX-induced reduction in the accumulation of Rho123, accompanied by the suppression of DOX-induced increases in reactive oxygen species levels. The pre- and co-administration of UDCA may be helpful in preventing the acquisition of multidrug resistance to DOX for the treatment of hepatocellular carcinoma.

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CASE REPORT

A pediatric case of hepatitis B virus subgenotype A2 in Japan

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Abstract Hepatitis B virus (HBV) has been classified into 10 major genotypes, and HBV genotypes C and B are found in the majority of Japanese patients. However, the prevalence of genotype A has been increasing in patients with chronic or acute hepatitis. Here we report a pediatric case of HBV subgenotype A2. A 2-year-old girl was referred to our hospital for liver damage caused by HBV infection. During the pregnancy, her father had developed acute sporadic hepatitis B. The child was born without any complications. She did not receive HBV vaccination at birth because her mother was negative for HBs antigen at the pre-delivery screening; however, her mother developed acute hepatitis B 2 months after delivery. At that time, HBs antigen was detected in the current patient. Phylogenetic full-length sequence analysis revealed HBV subgenotype A2. HBV sequencing was not performed for her parents; therefore, the intrafamilial transmission routes in these cases are unclear, although the authors speculate that, for the current patient, mother-to-child transmission may have occurred. This report illustrates the pitfalls of the selective vaccination strategy in Japan for preventing HBV infection. Universal vaccination to prevent HBV infection might be useful in Japan.

Keywords Hepatitis B virus · HBV genotype A · Universal vaccination

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Introduction

Hepatitis B virus (HBV) infection is a significant global health problem that affects 350 million individuals worldwide [1]. This infection follows a variety of clinical courses, such as development of the inactive carrier state, chronic active hepatitis and progression to cirrhosis and hepatocellular carcinoma [2]. HBV has been classified into ten major genotypes on the basis of 8 % divergence in the full-length sequence [3]. Each genotype has a unique geographic distribution and virological characteristics, and HBV genotypes C and B are found in the majority of Japanese patients [4, 5]. However, distributions of the HBV genotypes in Japan are changing due to the ease of international travel. Genotype A is found mainly in North Europe and Africa, and is subclassified into at least two subgenotypes, A1 (Asian/African type) and A2 (European type) [6]. However, the prevalence of genotype A in acute hepatitis B cases in Japan has recently been increasing, and many of these have progressed to chronic hepatitis [5, 7, 8]. As a result, the prevalence of genotype A has been increasing in adult patients with chronic or acute hepatitis

[9].The precise distribution of HBV genotype A in Japanese children is not known. Here we report a pediatric case of HBV subgenotype A2.

Case report

A 2-year-old girl was referred to our hospital for liver damage caused by HBV infection. Her parents both had histories of acute hepatitis B. During the pregnancy in December 2004, her father developed acute sporadic hepatitis B and was subsequently hospitalized for 20 days of treatment. The child was born without any complications in February 2005. In accordance with guidelines, she did not receive HBV vaccination at birth because her mother was negative for HBs antigen at the pre-delivery screening in May 2004. Her mother appeared to have jaundice 2 months after delivery, in April 2005, and was examined in the hospital. Although she had been negative for HBs antigen 11 months earlier, at the pre-delivery screening her HBs antigen was positive. Findings from her laboratory data included the following: alanine aminotransferase (ALT), 1163 U/L; asparatate aminotransferase (AST), 1553 U/L; total bilirubin, 4.9 mg/dL; and HBV, 6.3 log copies/ml. She was therefore diagnosed with acute hepatitis B. Because of this, the current patient received blood laboratory analysis, including testing for HBV infection, and was found to have serum ALT 32 U/L, AST 48 U/L, positive HBs antigen, and HBV 8.8 log copies/ml; therefore, she was also infected with HBV at this time. Her mother's HBs antigen became negative after 4 months and the HBs antibody became positive after 1 year.

At the first visit to our hospital in May 2007, positive results were obtained for the current patient for HBs antigen, HBe antigen and HBV DNA. Nested polymerase chain reaction (PCR) analysis and direct sequencing of full-length HBV was performed by the overlapping fragments method, as reported previously, but with modifications [10]. Phylogenetic analysis of the full-length sequence by the neighbor-joining method, as shown in

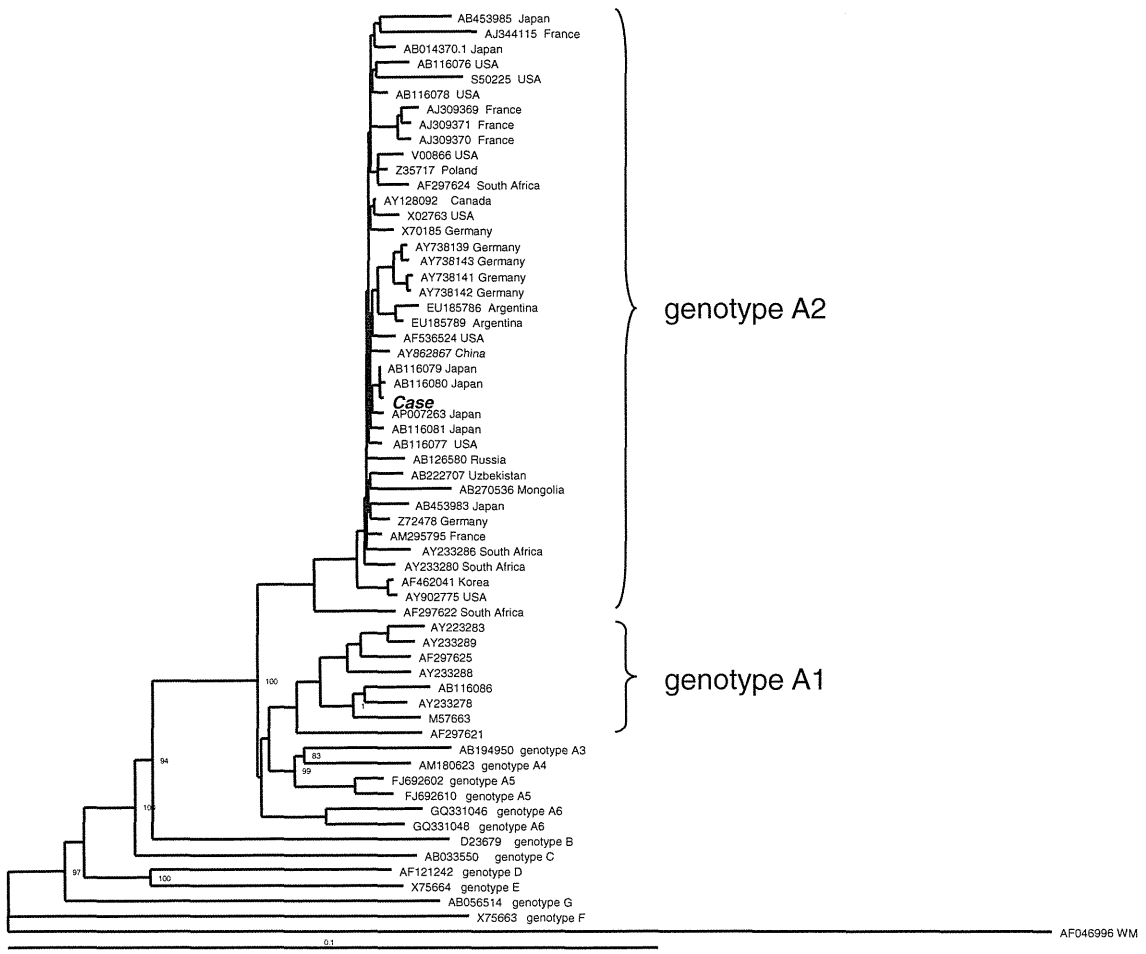

Fig. 1 Results of phylogenetic analysis of full-length HBV sequences from the current patient and 60 reference strains from a database, shown by accession number and country of origin. Phylogenetic analysis was performed by the neighbor-joining method, with Woolly monkey HBV (AF046996) as the out-group. The *scale bar* indicates genetic distance

Fig. 1, revealed the presence of subgenotype A2, and the fact that it was genetically related to a strain reported in Tokyo, Japan. The HBe antigen subsequently became negative in August 2010, and HBe antibody become positive in January 2011. The HBs antigen has been positive and HBV DNA has continued to be positive at 3.0 log copies/ml, as determined by real-time PCR. The liver enzymes remain slightly elevated (AST, 43 U/L; ALT, 31 U/L; γ-glutamyltranspeptidase, 115 U/L). As chronic hepatitis B is defined as HBs antigen positivity and ALT abnormality for more than 6 months, this clinical course has led to a current diagnosis of chronic hepatitis B.

Discussion

The prevalence of genotype A has been increasing in patients with chronic hepatitis, as well as in those with acute hepatitis [5, 7–9, 11]. Komatsu et al. [12] reported intrafamilial transmission of HBV subgenotype A2 in Japan. This case series included a 1-year-old boy; all genotyping data was confirmed by full-length sequence analysis of HBV from all cases. These data indicated that the HBV genotype A was widespread throughout Japan. In the current case, HBV sequencing and genotyping were not performed for the parents; the intrafamilial transmission modes therefore remain unclear. Based on the time course, the authors speculate that the father was originally infected, leading to transmission to the mother, and subsequently to mother-to-child transmission perinatally; however, horizontal transmission remains a slight possibility. Prevention of perinatal transmission by immunoprophylaxis with hepatitis B immunoglobulin and vaccine has decreased HBV infection in Japan, because the majority of these infections have involved genotypes B and C [13]. In most countries, universal vaccination is performed to prevent HBV infection, but in Japan, only high-risk groups, including healthcare workers and household contacts of HBV carriers, receive prophylactic HBV vaccination [14]. This different approach has been based on the geographic distribution of HBV genotypes and clinical characteristics. However, recently, frequencies of strains that were previously rare in Japan have been increasing among HBV-infected Japanese patients [5, 7–9, 11, 12]. In the past, prevention of perinatal transmission was satisfactorily achieved by targeted control of HBV infection; but this strategy now appears insufficient for prevention of horizontal transmission of HBV genotype A, which results in a higher rate of progression to chronic hepatitis when compared to genotypes B and C. This case illustrates a key disadvantage of the current strategy in Japan for prevention of HBV infection. Universal vaccination to prevent HBV infection might be useful in Japan.

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Original Article

Pegylated interferon monotherapy in patients with chronic hepatitis C with low viremia and its relationship to mutations in the NS5A region and the single nucleotide polymorphism of interleukin-28B

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Aim: Previous studies have suggested that patients with chronic hepatitis C with a low pretreatment hepatitis C virus (HCV) level have a high sustained virological response (SVR) rate, and that there would be a subpopulation of patients in which HCV can be eradicated with pegylated interferon (PEG IFN) alone without a decrease in SVR. However, the efficacy of PEG IFN monotherapy in patients with low HCV RNA levels is unclear. Several studies have reported that interferon sensitivity-determining region (ISDR) and the single-nucleotide polymorphism (SNP) of interleukin-28B (IL-28B) contribute to IFN response, but these relationships are controversial. The aim of this study was to determine whether the SNP of IL-28B (rs8099917) and amino acid substitutions in the ISDR among patients with low HCV levels affect the response to PEG IFN monotherapy.

Methods: One hundred and four patients with low-level HCV infection were studied. Low HCV level was defined as 100 KIU/mL or less.

Results: SVR was achieved in 94 patients (92.2%). HCV levels (≤50 KIU/mL) and ISDR (≥2 mutations) were associated with SVR on univariate analysis. The rates of SVR in the patients with IL-28B genotypes TT, TG and GG were 94.5%, 77.8% and 100%, respectively. The G allele tended to be associated with poor response to IFN therapy ($P = 0.0623$). On multivariate analysis, the ISDR was the factor predictive of SVR ($P = 0.004$).

Conclusion: The ISDR is significantly associated with a good response to PEG IFN monotherapy in patients with low HCV levels.

Key words: hepatitis C virus, interferon sensitivity-determining region, interferon, interleukin-28B, rapid virological response

INTRODUCTION

HEPATITIS C VIRUS (HCV) is a member of the Flaviviridae family and causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma (HCC) that easily progresses to end-stage liver disease.[1] Because 170 000 000 persons are infected with HCV worldwide, HCV infection is a significant global health problem.

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The current recommended therapy for patients with chronic hepatitis C is a combination of pegylated interferon (PEG IFN) and ribavirin and/or telaprevir or boceprevir.[2–6] HCV RNA levels, as well as genotypes, are an important factor associated with sustained virological response (SVR) to IFN therapy.[3,4] Patients with low HCV RNA levels have a high SVR rate, and even standard IFN monotherapy is useful for eradication of HCV in patients with low viral loads.[7–9] Several studies have succeeded in reducing the duration of treatment without risk of relapse.[10,11] Although patients with low HCV RNA have higher response rates to IFN treatment, not all patients achieve SVR. Other factors for improving the prediction of SVR in patients with low HCV RNA levels are needed. The predictive factors for SVR in patients with genotype 1b and high HCV RNA levels have been investigated, and several studies have shown that the single nucleotide polymorphism of interleukin-28B (IL-28B) and amino acid substitutions in the core and NS5A region affect the response to IFN therapy.[12–16] However, the predictive factors for SVR among patients with low HCV RNA levels treated with PEG IFN monotherapy have been unclear.

Hepatitis C virus consists of three structural proteins (core, envelope 1 and envelope 2) and six non-structural proteins (NS2 to NS5). HCV NS5A protein was reported to have a domain associated with IFN response. This domain in the region of HCV genotype 1b is closely associated with response to IFN therapy and is known as the IFN sensitivity-determining region (ISDR).[12,15–21] IFN acts to control replication of the virus by inducing the dsRNA-dependent protein kinase (PKR). The ISDR is located in the PKR-binding domain, is inhibited by PKR *in vitro*,[22] and is useful for prediction in patients with genotypes 2a, 2b and 3a.[23–28] Therefore, ISDR heterogeneity is an important factor that may affect response to IFN in patients with low HCV RNA levels. We hypothesized that ISDR heterogeneity could be predicted in patients with low HCV RNA levels in which HCV can be eradicated with PEG IFN-α alone without a decrease in SVR.

Not only genetic heterogeneity in the HCV genome but also host genetics contribute to IFN treatment outcomes. Therefore, several studies were performed to understand the host factors associated with IFN responsiveness; these showed that IL-28B polymorphisms are strongly associated with response to PEG IFN and ribavirin combination therapy in patients with genotype 1b and high viral load.[13,14,16,29] However, the associations between ISDR and IL-28B and the effects of PEG IFN-α monotherapy in patients with low HCV RNA levels are not well known.

The aim of the present study was to determine whether genomic heterogeneity of the ISDR and the SNP of IL-28B among patients with low HCV RNA levels affects the response to PEG IFN-α-2a monotherapy.

METHODS

A TOTAL OF 295 patients with chronic hepatitis C were treated by PEG IFN-α-2a monotherapy at Nagoya University Hospital and Affiliated Hospitals; 104 patients with low HCV RNA levels were selected for this study. The patients consisted of 62 men and 42 women with a mean age of 55.1 years (range, 19–78). All patients were positive for serum anti-HCV antibody by a commercial enzyme-linked immunosorbent assay (Dinabot, Tokyo, Japan) and for HCV RNA by a commercial polymerase chain reaction (PCR) (Roche Diagnostic Systems, Tokyo, Japan).

A low HCV level was defined as 100 KIU/mL or less, as previously reported.[4,7,9,11] No patient had hepatitis B surface antigen, co-infection with HIV, autoimmune disease or chronic alcohol abuse.

Schedule of IFN therapy

Patients received PEG IFN-α-2a (Pegasys Chugai-Roche, Tokyo, Japan) at a dose of 180 μg injected s.c. once per week for 24 or 48 weeks. The patients were allocated, at the discretion of the physician in charge, to a protocol lasting either 24 or 48 weeks. Laboratory tests and evaluations of adverse events were performed once per week during treatment.

The dose of PEG IFN-α-2a was reduced to 90 μg when clinically significant adverse events or laboratory abnormalities such as neutropenia (<750 cells/mm^3) or thrombocytopenia (<50 000 cells/mm^3) occurred. PEG IFN-α-2a was discontinued when neutropenia of less than 250 cells/mm^3 or a platelet count of less than 25 000 cells/mm^3 was seen.

Hepatitis C virus RNA in serum samples was examined at 4 weeks, at the end of IFN therapy, and at 6 months after the end of treatment (ETR). Serum was stored at −80°C for virological examination at pretreatment.

Patients who were persistently negative for serum HCV RNA and who had a normal serum alanine aminotransferase (ALT) level at 24 weeks after withdrawal of IFN treatment were considered to have SVR. Patients who were HCV negative at the ETR but returned to HCV

positive status after withdrawal of IFN were defined as virological relapsers. Patients who did not become HCV negative with IFN therapy were defined as non-virological responders.

This study was approved by the ethics committee of each institution involved. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Virological tests

Hepatitis C virus was genotyped by direct sequencing of the 5′-untranslated region and/or E1 regions, as described previously.[30,31] Genotypes were classified according to the nomenclature proposed by Simmonds *et al.*[32]

Nested PCR analysis and direct sequencing of the NS5A-ISDR were performed as previously reported for each genotype.[15,16,27,28] In brief, RNA was extracted from 140 μL serum using a QIAamp Viral RNA Kit (Qiagen, Valencia, CA, USA) and dissolved in 50 μL diethylpyrocarbonate-treated water. RNA (10 ng) was used for reverse transcription with oligo and random hexamer primers with an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). NS5A-ISDR was sequenced after amplification by nested PCR as previously described.[15,16,27,28]

The primers used were as follows: NS5A-ISDR of genotype 1b, sense 5′-TGGATGGAGTGCGGTTGCACA GGTA-3′ and antisense 5′-TCTTTCTCCGTGGAGGTGGT ATTG-3′; NS5A-ISDR of genotype 2a, sense 5′-ACGTCC ATGCTAACAGACCC-3′ and antisense 5′-GGGAATCT CTTCTTGGGGAG-3′; and NS5A-ISDR of genotype 2b, sense 5′-TCTCAGCTCCCTTGCGATCCTGA-3′ and antisense 5′-GATGGTATCGAAGGCTC-3′. Amplification conditions consisted of 10 min at 94°C, followed by 40 cycles of 94°C for 10 s, 55°C for 30 s and 72°C for 30 s in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems, Foster City, CA, USA). The second PCR was done using the following sets of primers: NS5A-ISDR of genotype 1b, sense 5′-CAGGTACGC TCCGGCGTGCA-3′ and antisense 5′-GGGGCCTTGGT AGGTGGCAA-3′; NS5A-ISDR of genotype 2a, sense from the first-round PCR and a new antisense primer 5′-CGAGAGAGTCCAGAACGACC-3′; and NS5A-ISDR of genotype 2b, sense 5′-AGCTCCTCAGCGAGCCA GCT-3′ and antisense 5′-GATGGTATCGAAGGCTC-3′. PCR products were separated 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 v1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems).

Genomic analysis

Detection of the SNP of IL-28B (rs8099917) was done by a real-time PCR system, as previously reported.[16] In brief, genomic DNA was extracted from 15 μL of whole blood using a commercial kit (QIAamp DNA Blood mini Kit; Qiagen) and dissolved in 50 μL diethylpyrocarbonate-treated water. DNA (1 ng) was used for PCR with primers and probes of commercial kit (Taqman SNP Genotyping Assays; Applied Biosystems). The SNP of IL-28B (rs8099917) was amplified, and the results were analyzed by real-time PCR in a thermal cycler (7300 Real time PCR System; Applied Biosystems).

Statistical analysis

Data are expressed as mean ± standard deviation. A paired Student's *t*-test or Fisher's exact test were used to analyze differences in variables. $P < 0.05$ was considered significant. Multiple logistic regression models were used to identify factors predictive of SVR. Statview ver. 5.0 software (SAS Institute, Cary, NC, USA) was used for all analyses.

RESULTS

Background

PATIENTS' CLINICAL CHARACTERISTICS are summarized in Table 1. HCV genotypes 1b ($n = 34$), 2a ($n = 58$), 2b ($n = 9$) and unknown ($n = 3$) were detected.

Table 1 Clinical characteristics at pretreatment

Clinical characteristics	$n = 104$
Age (years)	55.1 ± 12.5
Sex: male/female	62/42
AST (IU/L)	50.0 ± 28.2
ALT (IU/L)	62.7 ± 47.3
Platelet count (10^4/uL)	18.4 ± 5.7
HCV RNA level (KIU/mL)	36 (1.6–100)
HCV genotype (1b/2a/2b/unknown)	34/58/9/3
IFN length (weeks) (24/48/<17)	49/45/10
Body mass index	22.7 ± 3.2

Data are expressed as mean ± standard deviation.
HCV RNA level was shown by median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IFN, interferon.

Table 2 Virological response in each group

(a) Virological response according to durations of IFN therapy				
	Overall ($n = 102$)	24W ($n = 48$)	48W ($n = 45$)	<17W ($n = 9$)
RVR	81.4% ($n = 83$)	87.5% ($n = 42$)	73.3% ($n = 33$)	88.9% ($n = 8$)
ETR	100% ($n = 102$)	100% ($n = 48$)	100% ($n = 45$)	100% ($n = 9$)
SVR	92.2% ($n = 94$)	93.8% ($n = 45$)	91.1% ($n = 41$)	88.9% ($n = 8$)
(b) Virological response according to HCV genotypes				
	Overall ($n = 102$)	1b ($n = 32$)	2a ($n = 58$)	2b ($n = 9$)
RVR	81.4% ($n = 83$)	81.3% ($n = 26$)	81.0% ($n = 47$)	88.9% ($n = 8$)
SVR	92.2% ($n = 94$)	87.5% ($n = 28$)	93.1% ($n = 54$)	100% ($n = 9$)

ETR, end of treatment response; HCV, hepatitis C virus; IFN, interferon; RVR, rapid virological response; SVR, sustained virological response; W, weeks.

All patients had serum HCV RNA levels of 100 KIU/mL or less, and the median HCV RNA level was 36 KIU/mL.

One hundred and four patients were initially included in this study; 49 patients were treated with PEG IFN-α-2a for 24 weeks, and 45 patients were treated for 48 weeks. Ten patients withdrew from IFN therapy within 17 weeks, and two of these 10 patients could not be followed. The reasons for discontinuing therapy were fatigue ($n = 3$), depression ($n = 1$), rash ($n = 1$), appetite loss ($n = 1$), liver failure ($n = 1$) and unknown ($n = 3$). The two patients who withdrew from follow up were excluded from the analysis, and the remaining 102 patients were followed for 6 months after the ETR.

Virological response

Virological response is shown in Table 2. Rapid virological response (RVR), which was defined as negativity for HCV after 4 weeks of treatment, for the overall group, the 48 weeks' group, the 24 weeks' group and the under 17 weeks' group was 81.4% (83/102), 73.3% (33/45), 87.5% (42/48) and 88.9% (8/9), respectively. Virological response at the ETR was 100% among all patients. Finally, 94 (92.2%) of 102 patients achieved SVR.

There was no significant difference in virological response between patients treated for 24 weeks and those treated for 48 weeks. The virological response according to HCV genotype is shown in Table 2(b). Patients with genotype 1b had a lower SVR rate than genotypes 2a and 2b, but no significant differences in genotype were noted.

Genetic heterogeneity in NS5A-ISDR and response to IFN therapy

The prevalences of the number of amino acid substitutions in ISDR according to HCV genotypes are summarized in Figure 1. The ISDR were examined by direct sequencing, and classification involved counting the number of amino acid substitutions compared to consensus strains of each genotype, as previously reported.[15,24,27,28]

Interferon sensitivity-determining region sequences were obtained in 81 patients. Five patients did not have serum at pretreatment, and 16 patients could not be amplified by PCR. Sixty-one patients (84.7%) had one mutation or more. SVR according to the ISDR is shown in Figure 2. All patients with three or more mutations in the ISDR achieved SVR, but 18 (69.2%) of 26 patients with two or less mutations in the ISDR achieved SVR. Patients with two or less mutations in the ISDR were poor responders to IFN therapy.

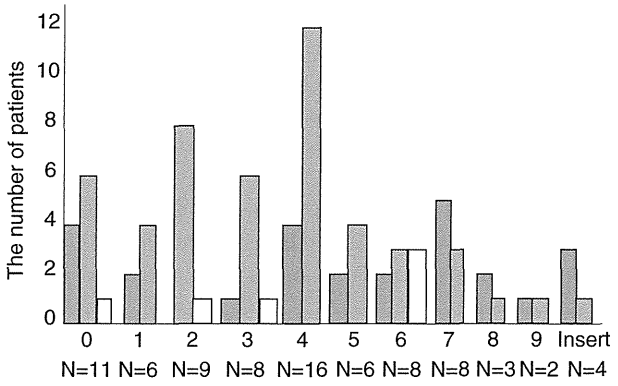

Figure 1 Number of amino acid substitutions in interferon sensitivity-determining region (ISDR) according to hepatitis C virus (HCV) genotypes. ■, HCV genotypes 1b; ▨, HCV genotypes 2a; □, HCV genotypes 2b.

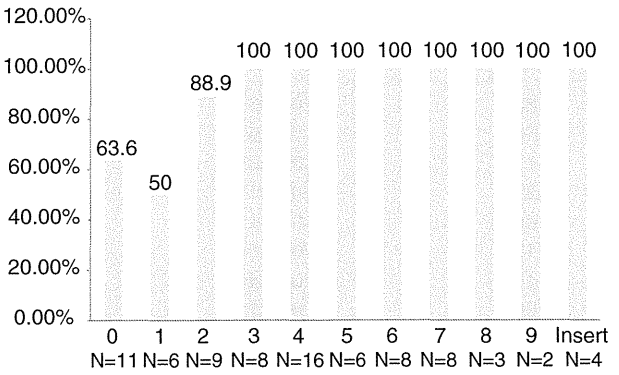

Figure 2 Sustained virological response (SVR) according to the number of amino acid substitutions in interferon sensitivity-determining region (ISDR).

Prevalence of the SNP of IL-28B (rs8099917) T (major allele) and G (minor allele) and response to IFN therapy

The frequencies of the IL-28B genotypes were: major homozygotes (TT), 73; heterozygotes (TG), 18; and minor homozygotes (GG), two. The rates of SVR in the patients with TT, TG and GG were 94.5% (69/73), 77.8% (14/18) and 100% (2/2), respectively. The SVR rate of patients with G allele of the IL-28B genotype was 80.0% (16/20), and that with T allele was 94.5% (69/73). Patients with T allele of the IL-28B genotype had a slightly higher SVR rate than did those with G allele, but there were no significant differences ($P = 0.0623$).

Analysis for factors predictive of SVR

The results of univariate analysis for factors predictive of SVR are shown in Table 3. HCV RNA levels were lower in patients with SVR than in those without SVR ($P = 0.0154$). SVR was achieved in 41.2% of patients with less than two mutations in the ISDR and 98.4% of patients with two or more mutations in the ISDR ($P = 0.0001$). HCV RNA levels and ISDR were associated with SVR on univariate analyses.

Results of multivariate analyses of factors predictive of SVR are shown in Table 4. Variables were recorded categorically as ordinal data. Background factors were age (<60 vs ≥60 years), sex (male vs female), platelet count ($<15 \times 10^4/mm^3$ vs $\geq 15 \times 10^4/mm^3$), HCV RNA level (<50 vs ≥50 KIU/mL), ALT levels (<70 vs ≥70 IU/L), aspartate aminotransferase (AST) levels (<60 vs ≥60 IU/L), HCV genotype (1 vs 2), ISDR (<2 vs ≥2 mutations), IL-28B (TT vs TG and GG) and RVR (yes vs no). As can be seen in Table 4, factors such as age, sex, platelet count, HCV RNA level, ALT levels, AST levels, HCV genotype, IL-28B and RVR did not have any effect on SVR. In contrast, the ISDR was the most influential factor.

DISCUSSION

THE HCV RNA level is one of the most important factors affecting response to IFN therapy. Patients with high HCV RNA levels respond poorly to IFN therapy, whereas patients with low HCV RNA levels have a high SVR rate to IFN therapy. Thus, most patients with low HCV RNA levels have achieved SVR, but other therapeutic options for patients who fail IFN therapy are needed. Several studies have attempted to reduce the duration of treatment, reduce the dose of IFN and/or ribavirin, or use standard IFN without risk of relapse.[8–10] The present study confirmed the high SVR rate (92.2%) in patients with low HCV RNA levels (≤100 KIU/mL)

Table 3 Univariate analysis: factors predictive of SVR

Factors	SVR ($n = 94$)	Non-SVR ($n = 8$)	*P*-value
Age (years)	54.6 ± 12.6	57.4 ± 8.8	0.5528
Sex: male/female	58/36	2/6	0.0619
ALT (IU/L)	63.2 ± 48.3	56.3 ± 32.5	0.7126
AST (IU/L)	50.7 ± 28.6	41.4 ± 21.6	0.4043
PLT ($\times 10^4/mm^3$)	18.5 ± 5.8	18.0 ± 5.0	0.8292
HCV RNA level (KIU/mL)	42.5 ± 34.8	75.0 ± 45.7	0.0154
HCV genotype: 1/2	29/63	4/3	0.4337
ISDR: <2/≥2	10/63	7/1	0.0001
IL-28B: TT/TG, GG	69/16	4/4	0.0623
RVR: yes/no	78/16	5/3	0.1661

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IL-28B, interleukin-28B; ISDR, interferon sensitivity-determining region; PLT, platelets; RVR, rapid virological response; SVR, sustained virological response.

Table 4 Multivariate analysis: factors predictive of SVR

Factors	*P*-value	Risk ratio	95% CI	
Age: <60 years	0.4556	2.837	0.183	43.891
Sex: male	0.8712	0.756	0.026	22.166
AST: <60 IU/L	0.7806	2.131	0.010	438.334
ALT: <70 IU/L	0.6063	0.239	0.001	55.563
Platelet count: $<15 \times 10^4$/uL	0.6873	0.463	0.011	19.680
HCV RNA: <50 KIU/mL	0.1046	13.170	0.585	296.318
Genotype: 2	0.1693	14.110	0.324	614.872
ISDR: <2	0.0074	0.004	0.001	0.235
IL-28B: TT	0.2684	5.978	0.252	141.852
RVR: yes	0.7495	1.756	0.055	55.696

95% CI, 95% confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IL-28B, interleukin 28B; ISDR, interferon sensitivity-determining region; RVR, rapid virological response; SVR, sustained virological response.

treated by PEG IFN-α-2a monotherapy. Although the effects of shortened treatment duration of PEG IFN-α with ribavirin for patients with low HCV RNA levels are unclear, PEG IFN-α-2a monotherapy could reduce the cost and adverse events of ribavirin while maintaining a high SVR rate. This treatment would be a good therapeutic option for patients with low HCV RNA levels. However, selection by HCV RNA level alone was insufficient to predict IFN responsiveness completely, and other factors would be necessary to improve the positive predictive values for SVR in patients infected with low HCV RNA levels.

Hepatitis C virus genotype is another major factor, in addition to HCV RNA levels, that is associated with response to IFN therapy. In the present study, the SVR rates of genotypes 1 and 2 were 87.5% and 94.0%, respectively. Patients infected with genotypes 2 had a slightly higher SVR rate than did those with genotype 1, but there were no significant differences in our small study. The difference in SVR according to genotype may exist, but HCV genotype did not have enough power to be a determinant of IFN response completely among patients with low HCV RNA levels because of the bias for HCV RNA levels. However, patients infected with low HCV RNA levels respond differently to IFN therapy, suggesting that an additional factor associated with resistance to IFN exists.

The heterogeneity of the HCV NS5A region is an important factor that may affect response to IFN in patients with HCV genotype 1b and was named the ISDR.[17] Mutations in the ISDR affect the interaction with PKR and may inhibit viral replication. Therefore, ISDR of other HCV genotypes, in addition to 1b, could be used as predictors of IFN responsiveness.[23–28] In the present study, it was hypothesized that the amino acid substitutions in the ISDR would explain differences in IFN resistance in patients infected with low HCV RNA levels. Therefore, the utility of substitutions of amino acids in the ISDR for predicting IFN responsiveness was investigated. The ISDR was the most influential factor for SVR on multivariate analyses. All patients with three or more mutations in the ISDR achieved SVR, and 18 of 26 patients with less than three mutations in the ISDR achieved SVR. Thus, patients with less than three mutations in the ISDR would be resistant to PEG IFN-α-2a monotherapy and may need to receive much more powerful treatment, even if they have low HCV RNA levels. The ISDR system could be used as a diagnostic tool to predict SVR in patients infected with low HCV RNA levels. An individualized treatment strategy based on HCV RNA levels and the ISDR in patients with chronic hepatitis C would be an important consideration to achieve optimal therapy and avoid unnecessary treatment.

Some studies of SVR to PEG IFN-α-2b and ribavirin and/or telaprevir combination therapy for chronic hepatitis C patients with genotype 1 and high viral load identified genetic variation near the IL-28B gene associated with IFN responsiveness.[13,14,16] However, the effects of genetic variation near the IL-28B gene on SVR in patients with low HCV RNA levels treated with PEG IFN monotherapy are unknown. Therefore, the utility of the SNP of IL-28B for predicting IFN responsiveness was investigated. Patients with IL-28B (rs8099917) genotypes TG and GG had a lower SVR rate than genotype TT, but no significant differences in genotype were found in this study. The SNP of IL-28B would be associated with the response to IFN, especially for poor responders, and

was partially associated with SVR in a study of patients with HCV genotype 2 who were treated with PEG IFN-α-2b and ribavirin.[13,14,16,33,34] The clear suggestion of a correlation between the SNP of IL-28B with IFN responsiveness would not be supported in patients with low HCV RNA levels because of the high SVR rate and predominant genotype 2.

Viral factors associated with SVR have been studied, and several regions, including 5′-untranslated region, core, E2, NS5A and NS5B, have been suggested to play important roles in IFN responsiveness.[14,16,35–38] Further studies need to investigate whether these other viral factors, especially interferon and ribavirin resistance-determining region of NS5A and core amino acid substitutions, among patients with low HCV RNA levels affect the response to PEG IFN monotherapy.

Hepatitis C virus RNA levels could be easy to measure using commercial kits and would be useful for clinical practice, but sequencing analysis, which involves much effort and cost, would be needed to characterize the ISDR. SVR was achieved in 95.1% of patients with lower HCV RNA levels (<50 KIU/mL) and 98.4% of patients with mutant type. ISDR was a better factor, but HCV RNA level might be used as a predictive factor instead of measurement of ISDR.

The definition of the low HCV RNA level that was related to a good response to IFN therapy has varied widely, from 100–600 KIU/mL.[7,9–11] Zeuzem *et al.* reported that 24 weeks of therapy with PEG IFN-α-2b plus ribavirin is insufficient for the treatment of patients with HCV genotype 1 and a HCV RNA level of 600 KIU/mL or less.[10] They suggested that patients with HCV RNA of 250 KIU/mL or less would have a good response to PEG IFN-α-2b and ribavirin combination therapy for 24 weeks. Most reports from Japan defined 100 KIU/mL as the cut-off level for low HCV levels and used standard IFN monotherapy.[4,7,9,11] The outcome that would maximize the efficacy of IFN therapy would depend on the relationships between the cut-off HCV RNA level and therapeutic regimens. The optimal cut-off level for low HCV levels and the matching therapeutic regimens are not well understood, and further studies are needed to clarify these issues.

Based on the SVR in patients receiving therapy for 24 weeks compared to those treated for 48 weeks, there was no difference in IFN responsiveness by duration in this small study. However, this study was not a randomized study. Further studies are needed to investigate the optimal duration of PEG IFN-α-2a monotherapy for patients with low HCV RNA levels.

Pascu *et al.* performed a meta-analysis for the correlation between SVR and ISDR in patients with HCV genotype 1b infection who received standard IFN therapy.[19] They found that 11 of 21 European patients with mutant type ISDR and HCV RNA levels of less than 6.6 log copies/mL achieved SVR, but 67 of 69 Japanese patients with mutant type ISDR and HCV RNA levels of less than 6.6 log copies/mL achieved SVR. The mode of HCV infection and geographical and racial differences would have effects on the prediction of SVR by ISDR.[39,40] As a result, the ISDR system is more suitable for predicting SVR in Asian than in European patients. Although validation of these observations in larger cohorts is required, mutations in the ISDR were useful for predicting the response to PEG IFN-α-2a monotherapy in patients with low HCV levels.

In conclusion, in patients with HCV infection, low HCV levels and more than two mutations in the ISDR are significantly associated with a good response to PEG IFN-α-2a monotherapy. An individualized treatment strategy based on HCV RNA levels and the ISDR in patients with chronic hepatitis C would be useful in clinical practice.

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Comparison of the Efficacy of Ribavirin Plus Peginterferon Alfa-2b for Chronic Hepatitis C Infection in Patients With and Without Coagulation Disorders

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Many patients with coagulation disorders are infected with hepatitis C virus (HCV) that advances to end stage liver disease, resulting in an increased number of deaths. The efficacy of ribavirin and peginterferon combination therapy for chronic HCV infection in patients with coagulation disorders has not been clarified fully. The aim of this study was to evaluate the efficacy and tolerability of combination therapy in this patient population compared with patients who are infected with HCV and do not have coagulation disorders. A total of 226 consecutive chronic hepatitis C patients were treated with combination therapy and divided into two groups: patients with (n = 23) and without coagulation disorders (n = 203). Clinical characteristics, sustained virological response rates obtained by an intention-to-treat analysis, and combination therapy discontinuation rates were compared between the two groups. The sustained virological response rates did not differ significantly between patients with and without coagulation disorders (65.2% vs. 47.8% by intention-to-treat analysis). According to a multivariate analysis, age, alanine aminotransferase, gamma-glutamyltransferase, and HCV genotype were associated significantly with a sustained virological response, whereas whether a patient had a coagulation disorder did not affect the sustained virological response. In conclusion, combination therapy for chronic hepatitis C was comparably effective between patients with and without coagulation disorders and did not result in adverse bleeding. ***J. Med. Virol. 85:228–234, 2013.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: chronic hepatitis C; interferon; ribavirin; coagulation disorders; hemophilia

INTRODUCTION

Hepatitis C virus (HCV) infection is a widespread viral infection that often leads to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Until the 1980s, most patients with coagulation disorders became infected with HCV because of the extensive use of untreated factor concentrate. Some of these patients were infected with both hepatitis C and human immunodeficiency virus (HIV) [Brettler et al., 1990; Troisi et al., 1993; Yee et al., 2000; Franchini et al., 2001]. These patients with liver diseases and persistent abnormal transaminase progress to end stage liver disease, resulting in an increased number of liver disease-related deaths. In cases of co-infection with the HIV, the progression of liver disease is more rapid [Sanchez-Quijano et al., 1995; Soto et al., 1997; Benhamou et al., 1999; Ragni and Belle, 2001; De Luca et al., 2002] with a higher mortality rate than

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during HCV monoinfection [Darby et al., 1997; Yee et al., 2000]. The need for treating infection with HCV in patients with coagulation disorders is increasing worldwide.

Sustained virological responders who are negative for serum HCV RNA 6 months after the end of treatment with interferon (IFN) are likely to remain in virological and biochemical remission with histologic improvement [Marcellin et al., 1997; Shiratori et al., 2000]. In addition, IFN therapy reduces the risk of hepatocellular carcinoma among virological or biochemical responders [Imai et al., 1998; Ikeda et al., 1999; Yoshida et al., 1999]. Ribavirin is now used generally in combination with IFN or pegIFN to treat chronic hepatitis C and combination therapy is more effective than IFN monotherapy [Lai et al., 1996; McHutchison et al., 1998; Poynard et al., 1998; Manns et al., 2001].

Previous studies have investigated the efficacy of IFN monotherapy in patients with coagulation disorders and chronic hepatitis C [Makris et al., 1991], and the efficacy of combination therapy with ribavirin and PegIFN in patients with coagulation disorders [Fried et al., 2002a; Mancuso et al., 2006; Posthouwer et al., 2007]. However, there are no reported comparisons of this combination therapy between patients infected with HCV with and without coagulation disorders. In this study, the efficacy and tolerability of ribavirin plus pegIFN were evaluated retrospectively in patients with coagulation disorders and chronic hepatitis C and the results were compared with the responses of patients infected with HCV but without coagulation disorders.

MATERIALS AND METHODS

Patients and Methods

A total of 226 consecutive patients with chronic hepatitis C and a high viral load (serum HCV RNA levels greater than 100 kilo-international units [KIU]) were treated with a combination of pegIFN and ribavirin between December 2004 and March 2007 at Nagoya University Hospital and Ogaki Municipal Hospital. These patients included 23 patients with coagulation disorders (17 with hemophilia A, 4 with hemophilia B, and 2 with von Willebrand disease). All patients were under 75 years old, were anti-HCV antibody-positive, and had serum HCV RNA levels greater than 100 KIU/ml by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0; Roche Molecular Systems, Pleasanton, CA) within 12 weeks preceding the therapeutic period. Patients were excluded if they had pretreatment hemoglobin (Hb) levels <10 g/dl, tested positive for serum hepatitis B surface antigen, a history of drug addiction, alcohol abuse, autoimmune hepatitis, primary biliary cirrhosis, a serious psychiatric or medical illness, or were pregnant. To exclude patient bias, only complete cohorts from each hospital were enrolled. HCV genotypes were determined by PCR using genotype-specific primers [Okamoto et al., 1994; Simmonds et al., 1994].

All patients were treated with 1.5 μg/kg of pegIFN α-2b (Peg-Intron®, MSD, Tokyo, Japan) once weekly for 24 weeks in patients infected with HCV genotype 2 or 3 and for 48 weeks in patients infected with HCV genotype 1 or 4. For the 17 patients infected with HCV genotype 1, the treatment duration was extended to 72 weeks because of higher efficacy compared to that obtained after 48 weeks of treatment, but only in cases in which HCV RNA was positive at 12 weeks and negative at 24 weeks from the start of therapy. Treatment was discontinued when a patient's Hb concentration fell below 8.5 g/dl because of drug-induced hemolytic anemia or when a patient's white blood cell count fell below 1,000/mm^3, neutrophil count fell below 500/mm^3, or platelet count fell below 50,000/mm^3. Some patients discontinued treatment because the virus could not be eradicated after 24 weeks, as determined by the physician. The pegIFN alfa-2b dose was reduced to 50% of the assigned dose when the white blood cell count was below 1,500/mm^3, the neutrophil count below 750/mm^3 or the platelet count below 8,000/mm^3. Oral ribavirin (Rebetol®, MSD, Tokyo, Japan) was administered for the same duration as pegIFN at 600 mg/day for patients who weighed 60 kg or less, 800 mg/day for those who weighed more than 60 kg but less than 80 kg, and 1,000 mg/day for those who weighed more than 80 kg during the treatment period. The ribavirin dose was reduced by 200 mg/day when the patient's Hb concentration fell below 10 g/dl because of drug-associated hemolytic anemia. Ribavirin was discontinued when pegIFN therapy was discontinued. Informed consent was obtained from each patient and the study was performed in accordance with the 1975 Declaration of Helsinki.

Liver Histology

Pretreatment liver biopsy specimens were classified based on a fibrosis scale of F0 to F4 (F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis) and in terms of necroinflammatory activity on a scale of A0 to A3 (A0, no histological activity; A1, mild activity; A2, moderate activity; and A3, severe activity) [Bedossa and Poynard, 1996; Fried et al., 2002b]. In patients with coagulation disorders, a liver biopsy was performed using factor concentrate, provided the patients gave informed consent.

Assessment of Efficacy

The virological response was assessed by a qualitative HCV RNA assay with a lower sensitivity limit of 100 copies/ml (Amplicor HCV version 2.0; Roche Molecular Systems). According to the qualitative HCV RNA results, responses were defined as a sustained virological response if no HCV RNA was detected at the end of the 24-week follow-up period after the treatment was completed. A patient was considered to have an end of treatment virological response if no HCV RNA was detected at the end of treatment.

Comparison of Characteristics and Treatment Efficacy Between Patients With and Without Coagulation Disorders

Sex ratio, age, body weight, body mass index (BMI), baseline serum alanine aminotransferase (ALT) levels, gamma-glutamyltransferase (GGT), pretreatment Hb level, platelet counts, HCV genotype and viral load, histologic activity, and fibrosis were compared between patients with and without coagulation disorders. The sustained virological response rates obtained by an intention-to-treat analysis and per-protocol analysis, ribavirin and pegIFN dose reduction rates, and combination therapy discontinuation rates were compared between the two groups. The end of treatment virological response rate was obtained by intention-to-treat and per-protocol analyses and then compared between the two groups. Next, the variable accession method in a multivariate analysis was used to examine factors associated with a sustained virological response after combination therapy, including the following factors: sex, age, BMI, baseline serum ALT, GGT, platelet counts, genotype, HCV RNA concentration, and presence of a coagulation disorder.

Because efficacy differed by the HCV genotype and the patient age, and since all coagulation disorder patients were male, the analysis focused on male, age-matched patients infected with HCV genotype 1. The characteristics and efficacy of treatment were compared in males, and age-matched patients with and without coagulation disorders who were infected with HCV-genotype 1.

Statistical Analysis

Values are expressed as the means ± SDs. Between-group differences in mean quantitative values were analyzed by Student's *t*-test, and differences in nonparametric data were analyzed by the Mann–Whitney *U*-test. Differences in proportions were examined by the Chi-squared test. Multiple logistic regression analysis was used to identify factors related to a sustained virological response. All statistical analyses were performed using SAS software (SAS Institute, Cary, NC). All P values were two-tailed, and $P < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

The patients included 127 men and 99 women aged 22–74 years (mean ± SD, 54.7 ± 11.6). The mean age of patients without coagulation disorders was 56.3 ± 10.9 years and most patients were in their 50s and 60s. In contrast, the mean age of patients with coagulation disorders was 41.5 ± 9.8 years with an age distribution ranging from 20 to 50 years. The clinical characteristics of the two study groups are shown in Table I. All patients with coagulation disorders in this study were male because of inherited, sex-linked hemophilia, and two patients in this study had male von Willebrand disease. Patients with coagulation disorders were significantly younger than patients without coagulation disorders ($P < 0.0001$). Although body weight was not different between the two groups, patients with coagulation disorders had a significantly lower BMI than patients without coagulation disorders. Patients without coagulation disorders were infected with HCV genotypes that are not unique to Japan, such as genotypes 1a, 3a, and 4a. Four patients with coagulation disorders were infected with human immunodeficiency virus and one of these patients had achieved a sustained virological response.

Response to Therapy

The ribavirin dose reduction rate tended to be higher in patients without coagulation disorders than in patients with coagulation disorders ($P = 0.0643$). The treatment discontinuation rate did not differ significantly between the two groups. As a result, the sustained virological response rate by an intention-to-treat analysis did not differ significantly between the

TABLE I. Clinical Characteristics of Patients Treated With Combination Therapy

	Total patients (n = 226)	Patients without coagulation disorders (n = 203)	Patients with coagulation disorders (n = 23)	*P* value
Sex ratio (male/female)	127/99	104/99	23/0	<0.0001
Age (years)	54.7 ± 11.6	56.3 ± 10.9	41.5 ± 9.8	<0.0001
Body weight (kg)	60.2 ± 11.1	60.5 ± 11.5	60.5 ± 8.1	0.9972
Body mass index	22.9 ± 3.1	23.1 ± 3.1	21.5 ± 2.5	0.0226
Baseline serum ALT (IU/L)	63.3 ± 56.8	60.9 ± 54.9	84.4 ± 69.1	0.0598
GGT (IU/L)	54.2 ± 63.9	51.4 ± 62.2	78.6 ± 74.4	0.0526
Hemoglobin (g/dl)	14.1 ± 1.3	14.1 ± 1.3	14.4 ± 1.3	0.2714
Platelets ($\times 10^4/\mu l$)	17.8 ± 5.2	17.7 ± 5.2	19.0 ± 5.6	0.2597
Genotype (1a/1b/2a/2b/3a/4a)	7/160/40/15/3/1	0/150/39/14/0/0	7/10/1/1/3/1	<0.0001
HCV RNA (KIU/ml)	1,898.0 ± 1,448.3	1,923.1 ± 1,464.5	1,676.6 ± 1,305.1	0.4404
Activity (A0/A1/A2/A3)	2/108/71/11	2/101/64/11	0/7/7/0	0.3442
Fibrosis (F0/F1/F2/F3)	17/104/49/22	16/97/45/20	1/7/4/2	0.5351

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HCV RNA, hepatitis C virus RNA; KIU, kilo-international units.

TABLE II. Efficacy of Combination Therapy

	Total patients (n = 226)	Patients without coagulation disorders (n = 203)	Patients with coagulation disorders (n = 23)	*P* value
SVR rate (intention-to-treat)	49.6 (112/226)	47.8 (97/203)	65.2 (15/23)	0.1130
SVR rate (per-protocol)	54.4 (111/204)	52.7 (97/184)	70.0 (14/20)	0.1405
ETR rate (intention-to-treat)	84.1 (190/226)	84.7 (172/203)	78.3(18/23)	0.4218
ETR rate (per-protocol)	89.1 (179/201)	89.6 (163/182)	84.2 (16/19)	0.4772
Ribavirin dose reduction rate	44.2 (100/226)	46.3 (94/203)	26.1 (6/23)	0.0643
PegIFN dose reduction rate	34.1 (77/226)	33.5 (68/203)	39.1 (9/23)	0.5891
Combination therapy discontinuation rate	9.8 (22/226)	9.4 (19/203)	13.0 (3/23)	0.5722

SVR, sustained virological response; ETR, end of treatment virological response; PegIFN, peginterferon.

two groups. The sustained virological response rate of patients with coagulation disorders by a per-protocol analysis was higher than that of patients without coagulation disorders, but there was no significant difference. In addition, based on both intention-to-treat and per-protocol analyses, the end of treatment virological response rate did not differ significantly between the two groups (Table II).

Factors associated with a sustained virological response in combination therapy were determined by a multivariate analysis. HCV genotype 1 and 4 versus 2 and 3 ($P = 0.001$, odds ratio 4.353 [95% CI, 1.810–10.469]), baseline serum GGT ($P = 0.003$, odds ratio 1.018 [1.006–1.030]), age ($P = 0.006$, odds ratio 1.053 [1.015–1.093]), and baseline serum ALT ($P = 0.014$, odds ratio 0.991 [0.983–0.998]) were associated significantly with a sustained virological response, but whether or not a patient had a coagulation disorder was not associated significantly with a sustained virological response.

Characteristics and Response of Male, Age-Matched Patients Infected With HCV Genotype 1

The clinical characteristics of the two study groups in the male, age-matched patients infected with HCV genotype 1 are shown in Table III. Body weight, BMI, and Hb levels were significantly lower in patients with coagulation disorders than patients without coagulation disorders ($P = 0.0003$, 0.0027, and 0.0103, respectively).

The treatment discontinuation rate of patients with coagulation disorders did not differ between the two groups. The sustained virological response rate by intention-to-treat and per-protocol analyses did not differ significantly between the two groups (Table IV). Factors associated with a sustained virological response in the male, age-matched, genotype 1 patients treated with combination therapy were determined by a multivariate analysis. BMI ($P = 0.036$, odds ratio 1.810 [1.041–3.145]) and baseline serum GGT ($P = 0.037$, odds ratio 0.981 [0.963–0.999]) were associated significantly with a sustained virological response, but whether or not a patient had a coagulation disorder was not associated significantly with a sustained virological response.

Adverse Events

The reasons for discontinuing combination therapy and the times at which the therapy was discontinued are shown in Table V. Once treatment was discontinued, therapy was not restarted even after the initial symptoms or illness disappeared. There were no bleeding episodes in the patients with coagulation disorders, including patients who received a liver biopsy.

TABLE III. Clinical Characteristics of Male, Age-Matched Patients With Genotype 1 Treated With Combination Therapy

	Total patients (n = 36)	Patients without coagulation disorders (n = 18)	Patients with coagulation disorders (n = 18)	*P* value
Age (years)	42.8 ± 8.0	44.9 ± 5.9	40.7 ± 9.3	0.1136
Body weight (kg)	66.1 ± 11.0	73.4 ± 9.3	60.4 ± 8.7	0.0003
Body mass index	22.7 ± 2.8	24.3 ± 2.3	21.4 ± 2.5	0.0027
Baseline serum ALT (IU/L)	69.8 ± 54.3	63.5 ± 31.7	76.2 ± 70.5	0.4919
GGT (IU/L)	72.7 ± 64.2	74.3 ± 71.1	71.2 ± 58.5	0.8869
Hemoglobin (g/dl)	14.9 ± 1.2	15.4 ± 1.0	14.4 ± 1.2	0.0103
Platelets ($\times 10^4/\mu l$)	19.3 ± 5.4	18.8 ± 4.5	19.8 ± 5.6	0.5773
HCV RNA (KIU/ml)	2,050.8 ± 1,273.4	2,322.8 ± 1,249.1	1,778.8 ± 1,273.5	0.2044
Activity (A0/A1/A2/A3)	0/12/11/0	0/6/5/0	0/6/6/0	0.6723
Fibrosis (F0/F1/F2/F3)	2/11/8/2	1/5/4/1	1/6/4/1	0.9392

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HCV RNA, hepatitis C virus RNA; KIU, kilo-international unit.

TABLE IV. Efficacy of Combination Therapy in Male, Age-Matched Patients With Genotype 1

	Total patients (n = 36)	Patients without coagulation disorders (n = 18)	Patients with coagulation disorders (n = 18)	*P* value
SVR rate (intention-to-treat)	58.3 (21/36)	61.1 (11/18)	55.6 (10/18)	0.7353
SVR rate (per-protocol)	69.0 (20/29)	64.7 (11/17)	75.0 (9/12)	0.5551
ETR rate (intention-to-treat)	77.8 (28/36)	83.3 (15/18)	72.2 (13/18)	0.4227
ETR rate (per-protocol)	93.1 (27/29)	88.2 (15/17)	100.0 (12/12)	0.2182
Ribavirin dose reduction rate	22.2 (28/36)	16.7 (3/18)	27.8 (5/18)	0.7175
PegIFN dose reduction rate	36.1 (13/36)	27.8 (5/18)	44.4 (8/18)	0.2979
Combination therapy discontinuation rate	5.6 (2/36)	0 (0/18)	16.7 (3/18)	0.0704

SVR, sustained virological response; ETR, end of treatment virological response; PegIFN, peginterferon.

DISCUSSION

A previous randomized trial in patients infected with HCV with inherited bleeding disorders showed that the sustained virological response rate improved significantly for patients who were treated with IFN and ribavirin compared to those treated with IFN alone [Fried et al., 2002a]. In addition, both chronic hepatitis C patients with and without coagulation disorders responded similarly to pegIFN and ribavirin combination therapy [Franchini et al., 2006; Posthouwer et al., 2006]. However, the efficacy and tolerability of this combination therapy differed based on the HCV genotype as well as the age, gender, and race of the patients; therefore it is difficult to compare patients with and without coagulation disorders under the same conditions. No report has examined that patients infected chronic hepatitis C with and without coagulation disorders at the same institution and during the same observation period. In addition, there are no reports on the efficacy of combination therapy in patients with chronic hepatitis C with and without coagulation disorders in age-matched patients infected with HCV genotype 1. Therefore, a retrospective study was conducted to evaluate the efficacy and tolerability of ribavirin plus pegIFN in chronic hepatitis C patients with and without coagulation disorders. In the per-protocol analysis, there were no significant differences, but the sustained virological response rate was higher in patients with coagulation disorders than in patients without coagulation disorders. Mancuso et al. [2006] reported that combination therapy with pegIFN alfa-2b plus ribavirin is highly efficacious in hemophiliacs with chronic hepatitis C. In an overall analysis, patients with coagulation disorders had a lower mean age than patients without coagulation disorders. In addition, the BMI of the patients with coagulation disorders was lower than that of patients without coagulation disorders. A multivariate analysis showed that the HCV genotype, baseline serum GGT, age, and baseline ALT were factors associated significantly with a sustained virological response and whether patients had coagulation disorders was not associated with a sustained virological response. Age, especially younger than 40 years old, was a good predictive factor for a sustained virological response, as was reported previously [Poynard et al., 2000; Fried et al., 2002b].

TABLE V. Reasons for Discontinuing Combination Therapy

Reason	Number	Weeks after starting treatment
Patients with coagulation disorders		
Peritonitis due to appendicitis	1	16
Pneumoniae	1	18
No HCV eradication	3	24, 28, 29
IDDM	1	44
Patients without coagulation disorders		
Fatigue	5	1, 2, 4.9, 19
Bleeding from duodenal varies	1	8
Dizziness	1	12
Palpitation	1	13
Cholecystitis	1	16
Symptom of Parkinson's disease	1	16
Fundal hemorrhage	1	17
Hepatocellular carcinoma	2	19, 21
Suspicion of Interstitial pneumonia	1	20
Gastric cancer	2	21, 36
Self-discontinuation	1	24
Neutropenia	1	25
Eruption	1	25
No HCV eradication	7	24, 25, 25, 27, 28, 29, 29

These results suggest that male patients who are infected with HCV genotype 1 and have coagulation disorders will have a higher sustained virological response than patients without coagulation disorders, if the coagulation disorder patients do not discontinue treatment. However, these results do not account for the differences in age. Therefore, male, age-matched patients infected with HCV genotype 1 were evaluated. The characteristics that differed between patients with and without coagulation disorders were body weight, BMI and baseline Hb levels.

In male, age-matched patients infected with HCV genotype 1, the sustained virological response rate based on both intention-to-treat and per-protocol analyses was not different between patients with and without coagulation disorders.

Using a multivariate analysis, whether patients had coagulation disorders was not associated significantly with a sustained virological response. Only BMI and GGT were identified as factors associated with a sustained virological response to combination therapy in male, age-matched patients infected with HCV genotype 1. A previous report showed that GGT levels may represent a surrogate marker of tumor necrosis factor-alpha expression in the liver and explain the importance of serum analyses to in predict the treatment outcome [Taliani et al., 2002]. Several studies revealed that GGT is one predictor of a sustained virological response [Taliani et al., 2002, 2006; Villela-Nogueira et al., 2005]. In western countries, obesity and a high BMI are associated with the absence of a sustained virological response to combination therapy of pegIFN or IFN with ribavirin [Bressler et al., 2003; Camma et al., 2004]. However, in Japan, most of the patients who are treated with combination therapy are not obese and have lower BMIs than patients in western countries. In this population, the mean BMI was 22.7 ± 2.8. In this low BMI population, a higher BMI would be associated with a sustained virological response. However, the reason why a low BMI is associated with the absence of a sustained virological response has not elucidated.

Adverse effects are thought to increase in patients with coagulation disorders; however, there was not a significant difference in adverse effects necessitating discontinuation of pegIFN and ribavirin between patients with and without coagulation disorders (13.0% vs. 9.4%). In addition, severe adverse effects and bleeding adverse effects were not associated with coagulation disorders. A previous report showed that IFN and ribavirin combination therapy may reduce the use of clotting factors in hemophilia patients with chronic hepatitis C [Honda et al., 2005; Yamamoto et al., 2006]. Ribavirin may reduce the side effect of bleeding during combination therapy. In this study, patients with coagulation disorders did not experience an adverse effect of bleeding.

In conclusion, treatment of chronic hepatitis C with combination therapy was effective comparably between patients with and without coagulation disorders and there were no adverse effects of bleeding.

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