and LDLR expression was enhanced in response to inhibition of cholesterol synthesis, whereas EPA decreased the expression of SREBP-1c and LDLR, by which fatty acid synthesis and cholesterol uptake might be lowered (Fig. 4C). Of note, although pitavastatin alone enhanced the expression of LDLR, the enhancement was abolished by addition of EPA (Fig. 4C). This effect of EPA indicates the clinical significance of the add-on therapy because LDLR is known to be an important cellular factor that is required for cell entry/infection of HCV. EPA addition is expected to accelerate the antiviral effect of peg-IFN, ribavirin and pitavastatin through repression of HCV entry/infection as well as HCV replication. It has recently been reported that HCV particles are enriched in cholesterol and virion cholesterol is involved in HCV cell entry, depending on Niemann-Pick C1-like 1 (NPC1L1), which is an HCV cell entry factor as well as a cellular cholesterol uptake receptor [Yamamoto et al., 2011; Sainz et al., 2012]. The NPC1L1 may be amenable to therapeutic intervention.

The analysis of viral dynamics during add-on therapy indicated that early phase viral decline within the first 2 weeks influenced the achievement of sustained virological response in patients with major type variation (TT) but not in those with minor type variation (TG + GG; Fig. 3A,B). It cannot be explained clearly why high sustained virological response rates were obtained in patients infected with HCV-1b with minor type variation, regardless of poorer viral decline with add-on therapy. Although there is still no evidence, in patients with minor type variation, statins and EPA may show their effect in a later phase, and the EPA effect of impeding HCV entry/infection through suppression of LDLR expression may contribute partly to the achievement of sustained virological response.

In univariate analysis, addition of pitavastatin and EPA, as well as genotype TT of IL28B at rs8099917, was positively associated with sustained virological response in peg-IFN plus ribavirin combination therapy (Table III). However, this association disappeared in a multivariate analysis, and IL28B variation remained as an independent factor. One of the major reasons may be that, compared with the addition of pitavastatin and EPA, TT variation of IL28B in the profile of individuals has overwhelming weight for governing the effect of peg-IFN plus ribavirin combination therapy. In our study, SNP variation of ITPA (rs1127354) did not influence treatment outcome but the planned dose of ribavirin was maintained well in patients with minor type variation (non-CC), as reported in previous studies (Fig. 2).

In conclusion, the lipid modulators, pitavastatin and EPA, could enhance the efficacy of peg-IFN plus ribavirin combination therapy through their synergistic antiviral effect, particularly in patients infected with HCV-1b with an intractable IL-28B allele. Although the research is still in the preliminary stages, there is a possibility that addition of pitavastatin and EPA may be effective for HCV-1b with core 70 mutation, and may increase sustained virological response rates in patients treated with triple therapy of peg-IFN, ribavirin, and telaprevir.

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Serum albumin is present at higher levels in alcoholic liver cirrhosis as compared to HCV-related cirrhosis

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Abstract. Residual hepatic functional reserve in cirrhotic patients is generally evaluated by a multivariate scoring system (Child-Pugh classification), which includes serum albumin levels as a variable. However, several patients show discrepancies between serum albumin levels and the progression of liver fibrosis, especially those with alcoholic cirrhosis. To assess whether hepatic capacity of protein synthesis varies with the etiology of cirrhosis, serum albumin and cholinesterase levels, and prothrombin time were compared between alcoholic cirrhosis and hepatitis C virus (HCV)-related cirrhosis. To minimize the influence of malnutrition and extrahepatic platelet destruction, patients with hepatocellular carcinoma, uncontrolled diabetes, appetite loss and/or splenal longitudinal size >15 cm were excluded. The patients with compensated liver cirrhosis were divided into three groups as follows: alcohol$^+$/HCV$^+$ (alcohol + HCV group; n=31), alcohol$^-$/HCV$^+$ (HCV group; n=31) and alcohol$^+$/HCV$^-$ (alcohol group; n=27). These groups were adjusted with respect to age, gender, body mass index and platelet count. Serum albumin levels in the alcohol group were significantly higher than those in the HCV group, with a difference of approximately 0.5 g/dl in every class of platelet count. The correlation of the alcohol + HCV group was intermediate between the alcohol and HCV groups. On the other hand, the correlations between serum cholinesterase levels and platelet counts were similar among the three groups. The prothrombin time was also comparable among the groups. Accordingly, serum albumin levels were higher in patients with alcoholic cirrhosis and alcohol consumption should be carefully considered when evaluating hepatic functional reserve.

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Abbreviations: BMI, body mass index; ChE, cholinesterase; GGT, γ-glutamyl transpeptidase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ICG, indocyanine green; PT, prothrombin time

Key words: alcohol, cirrhosis, hepatitis C virus, alcoholic liver disease

Introduction

Accurate assessment of residual hepatic functional reserve is indispensable for selecting an adequate treatment for patients with liver cirrhosis, particularly for those with liver tumors. Several tests have been proposed for determining residual liver function; however, no single marker is entirely reliable for predicting residual function, since hepatocytes possess a wide array of different functions (1,2). Instead of using a single marker, scoring systems using several parameters have been developed for assessing hepatic functional reserve and stratifying the severity of liver cirrhosis. Currently, the Child-Pugh score is widely accepted as a method to assess liver function during chronic liver disease, mainly cirrhosis (3,4). The Child-Pugh scoring system employs five clinical measures; serum albumin and bilirubin, ascites, encephalopathy and prothrombin time (PT), while the etiology of cirrhosis is not considered. In other words, hepatic capacity of protein synthesis is regarded as an important aspect of the Child-Pugh scoring system, and the evaluation system works on the assumption that every parameter worsens in parallel according to the progression of liver fibrosis, irrespective of the etiology. However, in a previous report examining prediction factors for variceal hemorrhage, the form of varices, red color sign and alcoholism were independent risk factors, whereas Child-Pugh variables were not included as significant factors (5). The result indicates that for each cause of cirrhosis, the relationship between the degree of fibrosis and clinical findings, including the capacity for protein synthesis, may vary.

In our experience, certain cirrhotic patients unexpectedly show high serum levels of albumin despite advanced liver fibrosis. Certain patients with alcoholic cirrhosis who underwent liver resection for hepatocellular carcinoma (HCC) had severe post-operational liver failure, even though they were within the permissible range under pre-evaluation as Child-Pugh class A with more than 3.5 g/dl of serum albumin. This is perhaps because the score for evaluating residual liver function was overestimated due to their serum albumin levels. Therefore, in this study we assessed hepatic protein synthesis

Table I. Patient characteristics.

	Alcohol group (alcohol$^+$/HCV$^-$)	HCV group (alcohol$^-$/HCV$^+$)	Alcohol + HCV group (alcohol$^+$/HCV$^+$)
No. of patients	27	31	31
Age (years)	67.2±7.6	68.2±7.9	64.2±9.7
Gender (male/female)	3/24	4/27	4/27
BMI (kg/cm^2)	23.6±0.5	23.2±0.6	23.4±0.6
Albumin (g/dl)	3.7±0.1	3.2±0.1	3.6±0.1[a]
Bilirubin (mg/dl)	1.8±0.2	1.7±0.2	1.3±0.2
AST (U/l)	44.6±3.8[c]	73.1±5.9	74.3±6.6
ALT (U/l)	27.3±1.9[d]	57.6±5.3	63.4±5.8
LDH (U/l)	292.8±25.2	410.7±37.5	356.1±30.2
GGT (U/l)	195.8±34.0[b]	52.7±7.0	103.7±15.0[a]
ALP (U/l)	390.3±41.3	452.8±53.0	433.7±44.9
ChE (mg/dl)	103.3±9.1[b]	70.9±6.4	81.3±5.4
Cholesterol (mg/dl)	159.3±5.5[b]	136.3±4.7	141.9±4.8
PT (%)	64.7±4.2	68.1±2.8	66.2±4.1
WBC (/μl)	4,444.8±245.8[b]	3,647.7±221.0	3,637.7±199.4
RBC (/μl)	392.6±12.5	368.3±9.4	373.7±10.0
Hemoglobin (g/dl)	12.5±0.4	12.0±0.4	12.5±0.32
Platelet (/μl)	8.2±0.5	7.3±0.4	7.5±0.5

BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; GGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; ChE, cholinesterase; PT, prothrombin time; WBC, white blood cells; RBC, red blood cells. [a]P<0.01 vs. HCV group; [b]P<0.05; [c]P<0.01; [d]P<0.001 vs. alcohol + HCV group.

capacity, including serum albumin levels, in patients with alcoholic cirrhosis and compared them to those in hepatitis C virus (HCV) infection-induced cirrhotic patients, which is the most common cause of liver cirrhosis and HCC. Platelet count was employed as a marker for liver fibrosis, since it has been previously used for predicting the progression of fibrosis (6,7) and can be measured simply and non-invasively.

Materials and methods

From April 2000 to March 2006, 225 outpatients with compensated liver cirrhosis were seen at the Department of Hepatology and Pancreatology, Kyushu University Hospital, including 170 HCV-positive patients. We defined an alcoholic patient as one with a daily consumption of >80 g ethanol for at least 10 years. Prior to enrolling the patients, patients with HCC, uncontrolled diabetes (HbA1c >6.5%) or recent appetite loss within 1 month were excluded. Furthermore, patients who had a splenal longitudinal size of >15 cm were also excluded. This selection was required to minimize the influence of malnutrition and extrahepatic destruction of platelets. After the selection, 45 alcoholic patients and 88 non-drinkers were identified as HCV-positive patients, while 30 alcoholic patients and 12 non-drinkers were identified as HCV-negative patients. For the alcoholic patients, only those who ceased drinking for at least 2 months were enrolled.

Finally, 31 alcoholic patients with HCV infection were enrolled (alcohol + HCV group). Stratification of patients according to their platelet count showed that the counts were <5.0x10^4/μl in 4 patients, 4.9-6.4x10^4/μl in 7 patients, 6.5-7.9x10^4/μl in 7 patients, 8.0-9.4x10^4/μl in 6 patients, 9.5-10.9x10^4/μl in 3 patients and >11.0x10^4/μl in 4 patients. To set a similar background to that of the alcohol + HCV group, we randomly selected 31 age-, gender-, body mass index (BMI)- and platelet count-matched non-drinking HCV-positive patients (HCV group). Similarly, we enrolled 27 HCV-negative alcoholic patients (alcohol group). All enrolled patients were negative for hepatitis B virus, anti-nuclear antibody and anti-mitochondrial antibody. The study protocol was approved by the Ethics Committee of the Kyushu University Hospital.

All quantitative data are expressed as the means ± standard deviation. Differences between categorical variables were analyzed using the Chi-square test. The Student's t-test was used for continuous variables. We considered P-values <0.05 to denote statistical significance.

Results

Regarding the blood testing results, significant differences were found only for serum γ-glutamyl transpeptidase (GGT) and albumin levels between the HCV and alcohol + HCV groups (Table I). There were no significant differences between the groups in terms of serum cholinesterase (ChE) and PT, which are general indices of hepatic protein synthesis capacity as well as albumin. In the alcohol + HCV group, aspartate aminotransferase and alanine aminotransferase levels were significantly lower, and the levels of GGT, ChE, cholesterol and white blood cell counts were significantly higher compared to the alcohol group. Platelet counts had been adjusted among the three groups.

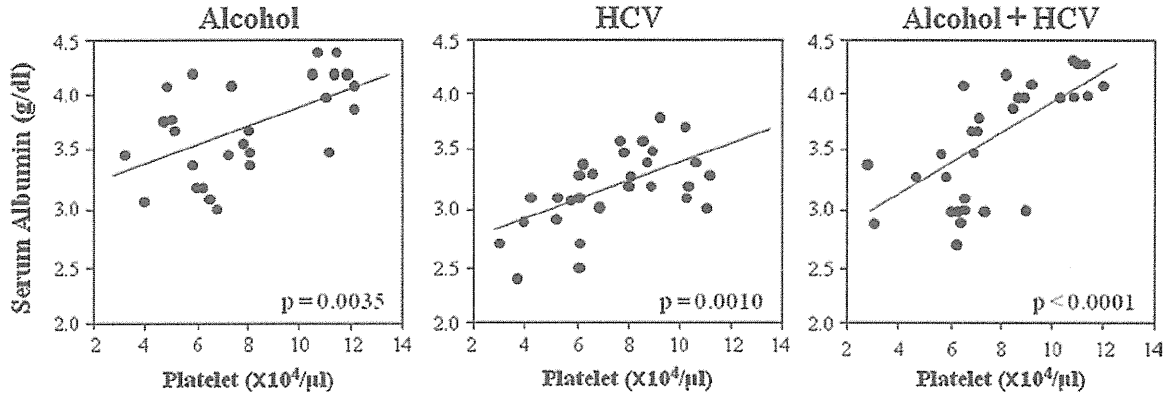

Figure 1. Correlation between serum albumin levels and platelet counts. Although the correlation was significant in each group, the levels of albumin in the alcohol group were always higher than in the HCV group. The approximate correlation line of the alcohol + HCV group was intermediate between the alcohol and HCV groups.

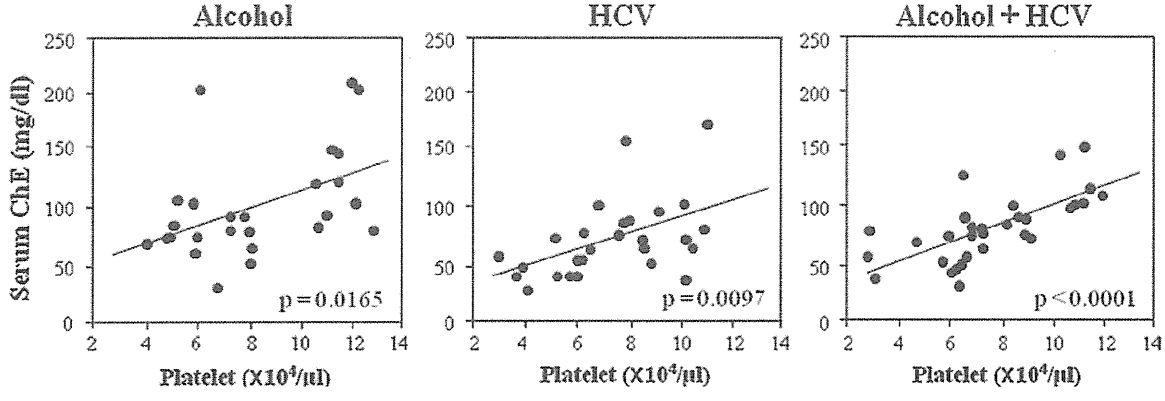

Figure 2. Correlation between serum cholinesterase levels and platelet counts. The correlations were almost similar in all three groups.

To clarify the relationship between the degree of liver fibrosis and capacity for protein synthesis, the correlation between serum albumin levels and platelet counts in the three groups was examined (Fig. 1). In each group, albumin levels significantly correlated with platelet counts. However, albumin levels were always higher in the alcohol group than in the HCV group, and the difference was ~0.5 g/dl irrespective of the platelet count. The approximate correlation line of the alcohol + HCV group was intermediate between that of the alcohol and HCV groups.

To determine whether the relationship of serum albumin with platelet counts was also true between other proteins produced in the liver and platelet counts, we examined the correlation between serum ChE and platelet counts in each group (Fig. 2). The correlation between ChE and platelet counts was significant but, interestingly, the levels and slope of the approximate correlation lines were almost similar among the three groups, in contrast to the relationship between albumin and platelet counts.

Discussion

This study showed that serum albumin levels were higher in patients with alcoholic cirrhosis than in those with cirrhosis caused by HCV irrespective of platelet count. These findings indicate that the hepatic capacity of albumin synthesis may be affected by the etiology, alcohol or HCV, in addition to the degree of liver fibrosis. Since protein synthesis is also influenced by nutritional state (8), careful establishment of the conditions for enrolling patients was required. We excluded patients with HCC, uncontrolled diabetes or appetite loss. We also confirmed that the BMI distribution did not differ between the groups. Although we used platelet count as a marker of the degree of liver fibrosis, other methods, such as indocyanine green (ICG) tests, hyaluronic acid and pathological findings based on liver biopsy, can also be used. Whichever parameter is used as a liver fibrosis marker, it should be recognized that each has potential weaknesses; for example, a decrease in platelet count can be overestimated in patients with marked splenomegaly (9), ICG tests show higher values when a portosystemic shunt exists (10), serum hyaluronic acid levels cannot differentiate fibrotic stage F1-3 (11) and liver biopsies are invasive and associated with a risk of sampling error (12,13). We employed the platelet count as it is a simple and non-invasive test, and is considerably reliable in patients at an advanced fibrotic stage (14). Patients with enlarged spleens (long axis diameter >15 cm) were excluded to reduce the influence of factors other than liver fibrosis and to increase its accuracy. Under these conditions, platelet counts probably reflect the degree of liver fibrosis, an assumption supported by the fact that platelet counts correlated significantly with serum albumin and ChE levels in all of the groups.

It is noteworthy that PT and serum ChE levels did not differ and the relationship between serum ChE and the platelet count was almost similar among the three groups, indicating that, irrespective of the etiology, hepatocytic products other than albumin decrease equally according to the progression of liver fibrosis. Practically, the evaluation of the hepatic functional reserve of the patients may not yield reliable results if only albumin values are considered and the etiology of cirrhosis is ignored. Our results raise questions as to why patients with alcoholic cirrhosis would have higher serum albumin levels than those with cirrhosis caused by HCV-infection. The role of HCV itself can be excluded as a significant difference was observed in albumin levels between the HCV and alcohol + HCV groups. Previous studies have suggested that alcohol can directly influence albumin synthesis. Annoni *et al* reported that patients with alcoholic cirrhosis showed significantly higher hepatic albumin mRNA levels than patients with a similar histological degree of cirrhosis due to viral infection (15). However, the alcoholic patients enrolled in the present study had stopped drinking at least 2 months prior to evaluation. Therefore, the possibility that alcohol or its metabolic products directly influenced the expression of albumin mRNA may be questionable. Other pathological differences may have contributed to the disparity in albumin synthesis, for example, the distinctive pathological characteristics of alcoholic cirrhosis, such as pericellular and perivenular fibrosis, Mallory bodies, steatosis and micronodular regeneration. Further investigations are required to determine the effect of these pathologies on albumin synthesis.

The present study demonstrated that the capacity for hepatic albumin synthesis in cirrhotic patients was differentially affected by the etiology of alcohol. Since serum albumin levels are commonly used as an important marker of hepatic functional reserve, ignoring the etiology of cirrhosis may lead to an incorrect evaluation. Serum albumin levels were present at higher levels and Child-Pugh scoring is likely to overestimate the residual hepatic functional reserve in patients with alcoholic cirrhosis. Therefore, alcohol consumption should be carefully considered when evaluating hepatic functional reserve.

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Review Article

Metabolic Disorders and Steatosis in Patients with Chronic Hepatitis C: Metabolic Strategies for Antiviral Treatments

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It has been reported that hepatitis C virus (HCV) infection is closely associated with hepatic metabolic disorders. Hepatic steatosis and insulin resistance are both relatively common in patients with chronic hepatitis C. Recent investigations suggest that HCV infection changes the expression profile of lipid-metabolism-associated factors in the liver, conferring advantages to the life cycle of HCV. Moreover, insulin resistance and steatosis are independent predictors of impaired response to antiviral treatment in chronic hepatitis C. In this paper, we summarize our current knowledge of hepatic metabolic disorders and describe how HCV leads to and exploits these hepatic disorders. We also discuss the clinical significance of insulin sensitizers used to improve insulin resistance and lipid modulators used to manage lipid metabolism as potential treatment options for chronic hepatitis C.

1. Introduction

Hepatic steatosis is a well-documented histological characteristic of chronic hepatitis C virus (HCV) infection [1]. Insulin resistance or impaired glucose metabolism, is linked to hepatic steatosis in patients with chronic hepatitis C (CH-C). It is widely considered that hepatic steatosis in patients with CH-C is caused by lipid metabolic disorders, in which insulin resistance plays an important role [2]. Fat accumulation promotes oxidative stress and inflammatory reactions. A considerable number of studies have also suggested that various HCV proteins lead to alterations in lipid synthesis, catabolism and transport. In particular, HCV core protein was reported to contribute to these metabolic changes and induce reactive oxygen species generation [3, 4]. Clinically, hepatic steatosis and insulin resistance in CH-C patients are associated with hepatic fibrosis, an increased frequency of hepatocellular carcinoma, and a poor response to pegylated interferon (peg-IFN) plus ribavirin combination therapy [5].

2. HCV Infection and Insulin Resistance

It has been reported that hepatic steatosis is correlated with viral load; approximately 50% of patients with CH-C have hepatic steatosis, which enhances disease progression [6, 7]. Recent studies have shown that, as in nonalcoholic fatty liver disease (NAFLD), insulin resistance and an increased fatty acid supply to the liver are important pathogeneses of steatosis in CH-C [8]. In CH-C patients, the occurrence of insulin resistance is independent of visceral adipose tissue and hepatic steatosis and irrespective of the HCV genotype [9]. In our experience, insulin resistance is frequently observed in nonobese patients, and 36.8% patients with CH-C had a homeostasis model assessment-insulin resistance (HOMA-IR) index ≥2.5 [10]. Even though the association between the severity of insulin resistance and HCV viral load or genotype is controversial, viral eradication by antiviral therapy actually improves insulin sensitivity [11–13]. Despite the close association between chronic HCV infection and the presence of insulin resistance, the pathogenic basis of this

interaction remains to be elucidated. Increasing epidemiological and experimental data suggest that the HCV core protein impairs insulin signaling, mostly by activating tumor necrosis factor α (TNFα) and members of the suppressor of cytokine signaling (SOCS) family [9, 14, 15].

SOCS proteins, which are induced by proinflammatory cytokines, induce proteasomal degradation of their target proteins, including insulin receptor substrate (IRS). Experimentally, upregulation of SOCS-1 and -3 in the liver leads to insulin resistance through several mechanisms, including degradation of IRS1 and IRS2 inhibition of insulin receptor kinase activity, and downregulation of IFN-associated innate immunity [16–18]. Activated TNFα inhibits tyrosine phosphorylation of IRS1 and IRS2, and impairs glucose transporter (GLUT)-4 translocation to the cell membrane, leading to insulin resistance and hyperinsulinemia, which can increase glycogenolysis and fatty acid synthesis [19, 20].

These changes may lead to hepatic steatosis by increasing the influx of free fatty acids via peripheral lipolysis, activation of lipogenesis-associated factors, reduced fatty acid oxidation, and decreased formation of very low-density lipoprotein (VLDL) [21]. IRS1 and IRS2 are closely linked to the regulation of glucokinase expression and lipogenic enzymes, such as sterol-regulatory element-binding protein 1c (SREBP-1c), respectively.

HCV infection, mainly through activity of the HCV core protein, decreases the expression and activity of peroxisome proliferator-activating receptor (PPAR)-α/γ in hepatocytes [22]. These effects may constitute strategies for viral survival and proliferation. PPARα and PPARγ transcriptionally regulate fatty acid β-oxidation and insulin sensitivity, respectively. Indeed, PPARγ agonists, thiazolidinediones, improve insulin sensitivity in CH-C patients. In our earlier study, we found that telmisartan, an angiotensin II receptor blocker and a potential partial PPARγ agonist, had significant therapeutic effects by attenuating insulin resistance and liver injury in patients with CH-C [10].

3. Lipid Metabolic Disorders in HCV-Infected Liver

A close association between HCV infection and lipid metabolism has been reported, and host metabolic factors and viral factors are likely to be involved in the pathogenesis of hepatic steatosis (see Figure 1). HCV core protein, which is localized to the membrane of lipid vesicles, induces hepatic fat accumulation by activating SREBP-1c [23, 24]. It also inhibits microsomal triglyceride transfer protein (MTP) activity, which is needed for VLDL assembly and excretion [25]. HCV infection decreases hepatic expression of PPARα, which negatively regulates fatty acid uptake and positively regulates β-oxidation, and promotes *de novo* lipid and cholesterol generation by enhancing the activities of SREBP-1 and -2 [24, 26].

In our evaluation of the expression of lipid metabolism-associated genes, the regulation of lipid metabolism was impaired in HCV-infected liver [27, 28]. The expression profiles revealed that HCV infection induced intrahepatic accumulation of cholesterol as well as triglycerides, resulting in a marked reduction of low-density lipoprotein receptor (LDLR) to decrease LDL-cholesterol uptake, and upregulated ATP-binding cassette G5 to increase cholesterol output. However, *de novo* cholesterol and fatty acid synthesis continued to increase, perhaps because of disrupted negative feedback pathways. This uncontrolled expression pattern is almost similar in NAFLD [29, 30]. However, HCV core protein interferes with the assembly and secretion of VLDL via inactivation of MTP, leading to hypobetalipoproteinemia, whereas, in NAFLD, MTP activity is enhanced and hyperlipidemia is common [8]. This expression pattern was also apparent in a preliminary evaluation of an HCV replicon system. Cholesterol is synthesized in hepatocytes through the mevalonate pathway, which is promoted by several enzymes, including HMG-CoA reductase (HMGR). Normally, the expression of LDLR and HMGR is regulated by the transcription factor SREBP-2 according to the intracellular cholesterol load. However, despite marked cholesterol accumulation, HMGR expression was greatly enhanced in HCV-infected liver [27, 28]. During cholesterol overload, the levels of cholesterol metabolites increase, including oxysterols, which act as agonistic ligands of liver X receptor-α (LXRα). These metabolites activate the LXRα-SREBP1c axis, which ultimately leads to activation of fatty acid synthesis. Notably, LXRα expression was also enhanced in HCV-infected liver [27, 28].

In addition to the core and nonstructural HCV proteins, the activation of cholesterol and fatty acid biosynthesis play a critical role in viral assembly, release, and infectivity [31–33]. Accordingly, viral interactions with the host's lipid metabolic pathways appear to be essential for the life cycle of HCV. Attachment of the virus to the cell surface LDLR represents the first stage of HCV entry into hepatocytes, and β-lipoproteins influence HCV proliferation [34, 35]. Serum HCV antigen levels are negatively correlated with serum β-lipoprotein levels [36]. The resulting lipid droplets supply lipoproteins and lipids and provide a site for viral assembly. These changes seem to be necessary or beneficial for HCV replication. The mevalonate pathway of *de novo* cholesterol synthesis, in which HMGR is a rate-limiting enzyme, is also responsible for the synthesis of farnesyl pyrophosphate and geranylgeranyl pyrophosphate that are essential for viral replication [37]. These molecules are needed to activate small GTPases, such as Rho and Ras. Therefore, HCV may need lipids not only as components of virus particles but also to modulate the host's cell signaling pathways.

4. Role of the Cannabinoid System in HCV-Infected Liver

Endocannabinoids, such as anandamide and 2-arachidonoylglycerol (2-AG), are synthesized from lipid precursors in cellular membranes and specifically target cannabinoid receptors (CB) 1 and CB2 [38]. Recent studies have suggested that the hepatic cannabinoid system is involved in the pathogenesis of NAFLD by activating CB1 and that steatogenic factors, such as a high-fat diet, induce the synthesis of

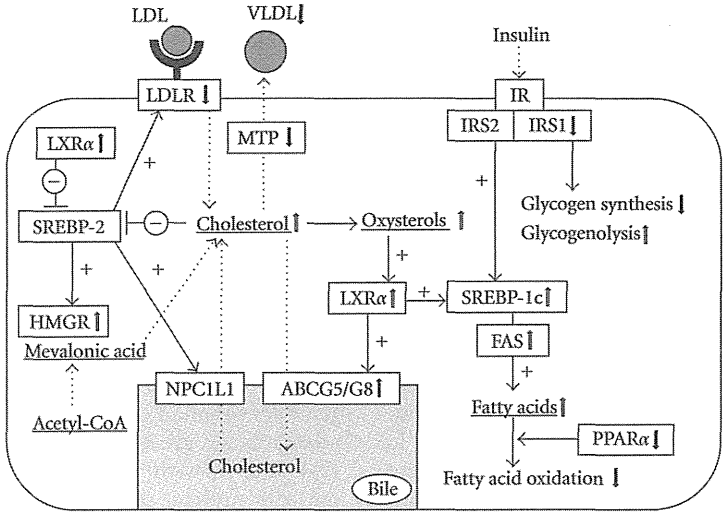

FIGURE 1: Expression profile of lipid metabolism-associated factors in chronic hepatitis C. ABCG5/G8, ATP-binding cassette G5/G8; FAS, fatty acid synthase; HMGR, HMG-CoA reductase; IR, insulin receptor; IRS, insulin receptor substrate; LDLR, LDL receptor; LXRα, liver X receptor α; MTP, microsomal triglyceride transfer protein; NPC1L1, Niemann-Pick C1-like 1; PPARα, peroxisome proliferator-activating receptor α; SREBP, sterol regulatory element-binding protein.

endocannabinoids and CB1 [39–41]. Although the signal transduction pathways have not been fully characterized, CB1 activation enhances the expression of several lipogenic factors, including SREBP-1c and fatty acid synthase (FAS) and downregulates factors involved in fatty acid oxidation, such as carnitine palmitoyltransferase I, resulting in steatosis and insulin resistance. Experimentally, steatogenic factors appear to activate CB2, but CB2 is mostly found in immune system cells. Therefore, CB2 activation may play a protective role against the inflammatory and fibrogenic responses in steatohepatitis [42, 43].

Because daily cannabis use was proposed as a risk factor for the severity of steatosis and progression of fibrosis in patients with CH-C [44], we determined the role of the hepatic cannabinoid system in HCV infection using HCV subgenomic replicon cells, which stably express viral nonstructural proteins (NS3, NS4A/4B, NS5A, and NS5B). Although the tested cannabinoid, anandamide, cannot be detected in culture medium, CB1 expression and triglyceride accumulation increased in replicon cells, as did the expression levels of several lipid synthesis-associated genes (SREBP-1c, FAS, and HMGR). IFNα treatment downregulates the expression of viral proteins and reduces triglyceride accumulation and gene expression of CB1, SREBP-1c, FAS, and HMGR. Meanwhile, treatment with a CB1 agonist increased, and a CB1 antagonist treatment decreased triglyceride accumulation in replicon cells.

These findings support the possibility that HCV infection activates the hepatic cannabinoid system and enhances steatotic changes in the liver. In healthy human liver, the hepatocytic expression of CB1 and CB2 is very low or even absent, as are endocannabinoid levels [45–48]. Marked upregulation of these receptors and endocannabinoid levels (anandamide and 2-AG) has been reported in the cirrhotic liver [45, 49–51]. Moreover, in acute liver damage, the expression of CB1 and CB2 is enhanced, and the degree and duration of inflammation may be an important factor for controlling CB1 expression or activation of the cannabinoid system. However, in a quantification assay using real-time polymerase chain reaction, CB1 gene expression was very low in liver samples from CH-C patients and healthy individuals (unpublished data). In patients with CH-C, more severe inflammation or fibrosis may be needed to activate the cannabinoid system.

As described above, there is a discrepancy between *in vitro* data in HCV replicon cells and findings in the liver of patients with CH-C. Therefore, it is questionable whether activation of the cannabinoid system significantly affects metabolic disorders in the HCV-infected liver. Of note, some researchers have proposed the existence of cannabinoid receptors other than CB1 and CB2 and endocannabinoids other than anandamide and 2-AG [52], although these have not yet been clearly detected. Therefore, still unknown cannabinoids and/or receptors may play a leading role in hepatic metabolic disorders in patients with CH-C.

5. Therapeutic Strategies Using Metabolic Modulators

Clinically, antioxidants, such as ursodeoxycholic acid and vitamin E, have been commonly used for NAFLD and CH-C patients as a liver supporting therapy. In many patients with insulin resistance, insulin sensitizers, such as metformin and thiazolidinediones, have shown improving effect of liver biochemistry. Nowadays, IFN-based radical antiviral treatments are generally accepted for patients with CH-C. Hepatic steatosis and insulin resistance are negative predictors for sustained virological response (SVR) in patients with

CH-C treated with peg-IFNα plus ribavirin combination therapy [53, 54]. In recent meta-analyses, HOMA-IR, a marker of insulin resistance, is negatively correlated with SVR, irrespective of viral genotype [55, 56]. Therefore, lifestyle modifications, such as weight reduction by exercise and nutritional management, are recommended to enhance the effects of antiviral treatments. Moreover, it is justifiable that the use of agents targeting insulin resistance and dyslipidemia can improve the SVR rate achieved with IFN-based antiviral treatments.

Insulin sensitizers, metformin and thiazolidinediones, may increase the response to antiviral treatments [57]. In a recent clinical trial of insulin-resistant patients with CH-C genotype 1, adding metformin to standard peg-IFNα plus ribavirin therapy improved insulin sensitivity. Metformin also tended to improve SVR, particularly in females, although a statistically significant difference was not seen compared with patients receiving placebo [58]. Meanwhile, the effects of pioglitazone on SVR in patients with CH-C and insulin resistance are controversial. Pioglitazone combined with peg-IFNα plus ribavirin therapy was used as retreatment and in treatment-naïve patients, but results of two pilot trials were disappointing [59, 60]. However, some reports have described that the addition of pioglitazone to standard therapy improves SVR and insulin sensitivity [61, 62]. This discrepancy may be explained, at least in part, by genotype dependency and host characteristics.

As described above, the synthesis of cholesterol and fatty acids is still activated in the liver of patients with CH-C, despite lipid overaccumulation. Therefore, correcting cholesterol and fatty acid synthesis by lipid modulators may help to reduce steatosis and improve SVR with antiviral treatments. Considering that cholesterol synthesis is enhanced in HCV-infected liver, it is plausible that HMGR inhibitors (statins) could have antiviral effects, because statins were recently reported to suppress HCV replication [63]. In fact, it was reported that statins do impede HCV replication by inhibiting host protein geranylgeranylation, and FBL2 has been identified as a geranylgeranylated cellular protein required for HCV RNA replication [64]. Retrospective analyses of patients with CH-C treated with peg-IFN plus ribavirin combination therapy have shown that serum cholesterol and the use of statins are positive predictors of SVR [65, 66]. In clinical trials, SVR was improved by adding fluvastatin or pitavastatin to peg-IFN plus ribavirin treatment [67–69]. Although antiviral activity has been experimentally demonstrated in most statins without pravastatin [63], a statin with a more activity may achieve better SVR rates. Of note, protease inhibitors, such as telaprevir, and statins taken together may raise the blood levels of statins and increase the risk of myopathy, kidney damage, and kidney failure. It was also reported that polyunsaturated fatty acids (PUFAs) inhibit HCV replication by a still unclear mechanism, independent of their roles in regulating lipogenesis and that eicosapentaenoic acid (EPA), an n-3 PUFA, inhibits HCV replication in the replicon system and suppresses SREBP-1c activity [70–72]. Additionally, administration of EPA allows maintenance of the original ribavirin dose in patients with CH-C during peg-IFN plus ribavirin combination therapy [73]. Using HCV replicon systems, it was reported that statins and EPA have suppressive effects against HCV replication and synergistic antiviral action with IFN [37, 70, 71, 74, 75].

Based on experimental and therapeutic evidence, concomitant administration of a statin and EPA with peg-IFN plus ribavirin therapy is pathophysiologically promising for patients with CH-C. Accordingly, we are now performing a clinical trial using a new antiviral strategy for patients with CH-C genotype 1b in which pitavastatin (2 mg/day) and EPA (1,800 mg/day) are added to standard peg-IFN plus ribavirin therapy. According to recent clinical studies of patients with CH-C genotype-1b, mutation of amino acids 70 and 91 in the core region of HCV-1b, as a virus-related factor, and genomic variation of the *IL28B* gene (rs8099917), as a host-related factor, are strong predictors of the outcome of peg-IFN plus ribavirin combination therapy [76–79]. Within the core protein, substitution of amino acid 70 seems to be more influential on the outcome than substitution of amino acid 91 [79–81]. At present, our trial has yielded several important findings (unpublished data). First, add-on pitavastatin and EPA therapy conferred significantly higher SVR rates than did standard therapy. Second, add-on therapy significantly improved SVR rates, particularly in patients with the minor variant (TG + GG) of *IL28B* (rs8099917), in whom SVR is expected to be poor. Of note, among patients treated with add-on therapy, genomic variation of *IL28B* still predicts clinical outcomes, because SVR rates were significantly higher in patients with the major variant (TT) than in those with minor variants. Third, mutation of core amino acid 70, which is a strong negative predictor of SVR in standard peg-IFN plus ribavirin therapy, did not diminish the outcomes of add-on therapy.

6. Conclusions

Steatosis and insulin resistance induced by HCV infection are, at least in part, critical factors for the progression of CH-C and can influence the outcome of antiviral treatments. Therefore, managing these metabolic disorders by administering insulin sensitizers and lipid modulators has been examined to increase the therapeutic response of standard treatments. In particular, concomitant treatment with pitavastatin and EPA may achieve considerable improvements in the efficacy of peg-IFN plus ribavirin combination therapy, particularly in patients with CH-C resistant to standard peg-IFN plus ribavirin therapy.

Conflict of Interests

The authors have no conflict of interests to declare.

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Special Report

A multicenter survey of re-treatment with pegylated interferon plus ribavirin combination therapy for patients with chronic hepatitis C in Japan

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Aim: This study aimed to clarify the factors associated the efficacy of re-treatment with pegylated interferon (PEG IFN) plus ribavirin combination therapy for patients with chronic hepatitis C who had failed to respond to previous treatment.

Methods: One hundred and forty-three patients who had previously shown relapse ($n = 79$), non-response ($n = 34$) or intolerance ($n = 30$) to PEG IFN plus ribavirin were re-treated with PEG IFN plus ribavirin.

Results: Twenty-five patients with intolerance to previous treatment completed re-treatment and the sustained virological response (SVR) rates were 55% and 80% for hepatitis C virus (HCV) genotype 1 and 2, respectively. On re-treatment of the 113 patients who completed the previous treatment, the SVR rates were 48% and 63% for genotype 1 and 2, respectively. Relapse after previous treatment and a low baseline HCV RNA level on re-treatment were associated with SVR in genotype 1 ($P < 0.001$). Patients with the interleukin-28B major genotype responded significantly better and earlier to re-treatment, but the difference in the SVR rate did not reach a significant level between the major and minor genotypes ($P = 0.09$). Extended treatment of 72 weeks raised the SVR rate among the patients who attained complete early virological response but not rapid virological response with re-treatment (72 weeks, 73%, 16/22, vs 48 weeks, 38%, 5/13, $P < 0.05$).

Conclusion: Relapse after previous treatment and a low baseline HCV RNA level have predictive values for a favorable response of PEG IFN plus ribavirin re-treatment for HCV genotype 1 patients. Re-treatment for 72 weeks may lead to clinical improvement for genotype 1 patients with complete early virological response and without rapid virological response on re-treatment.

Key words: chronic hepatitis C, pegylated interferon and ribavirin combination therapy, re-treatment

INTRODUCTION

PEGYLATED INTERFERON (PEG IFN) plus ribavirin combination therapy can show antiviral efficacy for patients with chronic hepatitis C (CH-C). However, a sustained virological response (SVR), which is defined as undetectable serum hepatitis C virus (HCV) RNA at 24 weeks after the treatment, remains at 50% for patients with HCV genotype 1 and 80% for those with HCV genotype 2 treated with PEG IFN plus ribavirin.[1–6] The number of patients who fail to achieve a SVR increases over time, requiring urgent action to eradicate HCV in them.

Recently, addition of the first-wave protease inhibitor telaprevir to PEG IFN plus ribavirin combination therapy, which has been reported to improve antiviral efficacy, has become commercially available, but this

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triple therapy increases side-effects, especially severe anemia and skin rash.[7–11] Second-wave protease inhibitors, such as TMC435, which not only improve antiviral efficacy but also decrease side-effects, have been developed and are undergoing clinical trials.[12] Also, IFN-free regimens, such as protease inhibitor and polymerase inhibitor combination therapy, have been developed.[13,14] In Japan, HCV carriers are increasing in an aging population, and large numbers of patients are ineligible for triple therapy with telaprevir due to potential anemia. That is why re-treatment with PEG IFN plus ribavirin is a possible choice for patients who failed to achieve SVR to previous antiviral therapy or patients ineligible for triple therapy with telaprevir who must wait until next-generation antiviral therapies, such as triple therapy with second-wave protease inhibitors or IFN-free regimens, become commercially available.

As for re-treatment with PEG IFN plus ribavirin, some studies have been reported but the subjects and treatment protocols were varied.[15–20] According to past reports, the previous treatment response is associated with the efficacy of the re-treatment[17,20] and the SVR rates in re-treatment ranged 4–23%.[16–18] Recently, host factors, such as single nucleotide polymorphisms (SNP) located near the interleukin (IL)-28B gene, and virus factors, such as the amino acid substitutions in the HCV core region, were revealed to have a strong impact on SVR in PEG IFN plus ribavirin combination therapy for naïve CH-C patients.[21–26] Moreover, response-guided therapy which extends treatment duration until 72 weeks for patients with a slow virological response can raise the SVR rate for naïve CH-C patients.[27–29] However, the value of IL-28B SNP has been uncertain in re-treatment and the most appropriate treatment duration in re-treatment is still unclear. Although it remains obscure which factors are associated with SVR in re-treatment with standard PEG IFN plus ribavirin therapy as pointed out above, some patients do respond to re-treatment and it is very important to be able to identify them. Such findings will be valuable for optimizing the antiviral treatment for CH-C patients by making it possible to decide which patients should be considered for re-treatment with PEG IFN plus ribavirin therapy and which should wait for next-generation antiviral treatment.

In the present study, we tried to determine which patients could benefit from re-treatment and to identify the factors associated with SVR in re-treatment, including the host genome SNP and treatment duration.

METHODS

Patients

THIS RETROSPECTIVE, MULTICENTER study was conducted by the Study Group of Antiviral Therapy for Difficult-to-Treat Chronic Hepatitis C supported by the Ministry of Health, Labor and Welfare, Japan. This study was conducted with 143 CH-C patients, 113 patients (genotype 1, $n = 86$; genotype 2, $n = 27$) who had previously completed PEG IFN-α-2b plus ribavirin combination therapy but had failed to attain SVR, and 30 patients (genotype 1, $n = 22$; genotype 2, $n = 8$) who had previously discontinued this combination therapy due to adverse events.

Treatment

For the previous treatment, patients had been treated with PEG IFN-α-2b (PEGINTRON; MSD, Whitehouse Station, NJ, USA) plus ribavirin (REBETOL; MSD). For re-treatment with PEG IFN plus ribavirin, patients were treated PEG IFN-α-2a (PEGASYS; Roche, Basel, Switzerland) plus ribavirin (COPEGUS; Roche) or PEG IFN-α-2b plus ribavirin. In principle, as a starting dose, PEG IFN was given once weekly at a dose of 180 μg of PEG IFN-α-2a and 1.5 μg/kg of PEG IFN-α-2b and ribavirin was given at a total dose of 600–1000 mg/day based on bodyweight (bodyweight, ≤60 kg, 600 mg; 60–80 kg, 800 mg; ≥80 kg, 1000 mg), according to the standard treatment protocol for Japanese patients and the decision of the investigator at the participating clinical center. Dose modification followed, as a rule, the manufacturer's drug information on the intensity of the hematological adverse effects.

Laboratory tests and virological assessment

Examination of peripheral blood, transaminase and the serum HCV RNA level were tested at the start of treatment, weeks 4, 12 and 24, end of treatment (EOT), and 24 weeks after the treatment. Sequences of the IFN-sensitivity determining region (ISDR) and the core region of HCV were determined at start of the previous treatment, and the number of mutations in the ISDR, the amino acid substitutions at core 70 and 91, glutamine (Gln) or histidine (His) at core 70 and methionine (Met) at core 91, were analyzed. Genetic polymorphisms located near the IL-28B gene (rs8099917) and ITPA gene (rs1127354) were determined. As for the IL-28B gene, homozygosity for the major sequence (TT) was defined as having the IL-28B major allele, whereas homozygosity (GG) or heterozygosity (TG) of the minor sequence was defined as having

the IL-28B minor allele. As for the ITPA gene, homozygosity for the major sequence (CC) was defined as having the ITPA major allele, whereas homozygosity (AA) or heterozygosity (CA) of the minor sequence was defined as having the ITPA minor allele. The serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test ver. 2.0 (detection range, 6–5000 KIU/mL; Roche Diagnostics, Branchburg, NJ, USA) or COBAS TaqMan HCV test (detection range, 1.2–7.8 $\log_{10}$ IU/mL) and qualitatively analyzed using the COBAS AMPLICOR HCV test ver. 2.0 (lower limit of detection, 50 IU/mL). When the serum HCV RNA level quantified by the COBAS TaqMan HCV test was less than 1.7 $\log_{10}$ IU/mL, which was equivalent to 50 IU/mL of HCV RNA, that case was judged as HCV RNA negativiation against the lower limit of detection of the COBAS AMPLICOR HCV test.

Definition of virological response

A rapid virological response (RVR) was defined as undetectable serum HCV RNA level at week 4, partial early virological response (p-EVR) as a more than 2-log decrease in the HCV RNA level at week 12 compared with the baseline, complete EVR (c-EVR) as undetectable serum HCV RNA at week 12, late virological response (LVR) as detectable serum HCV RNA at week 12 and undetectable at week 24, and SVR as undetectable serum HCV RNA at 24 weeks after the treatment. Relapse was defined as undetectable serum HCV RNA at the EOT but a detectable amount after the treatment. Patients without p-EVR or without clearance of HCV RNA at week 24 were considered to be showing non-response (NR), and treatment was stopped in both the previous treatment and this re-treatment. A patient who attained HCV RNA negativiation during the re-treatment continued to be treated for 48 weeks or 72 weeks according to response-guided therapy or the decision of the investigator at the participating clinical center.

Statistical analysis

Baseline data of the patients are expressed as means ± standard deviation or median values. In order to analyze the difference between baseline data or the factors associated with SVR, univariate analysis using the Mann–Whitney *U*-test or χ^2-test and multivariate analysis using logistic regression analysis were performed. A two-tailed *P*-value of less than 0.05 was considered significant. The analysis was conducted with SPSS ver. 17.0J (IBM, Armonk, NY, USA).

RESULTS

THE PATIENT FLOW in this study is shown in Figure 1. Among the patients who had previously discontinued PEG IFN-α-2b plus ribavirin combination therapy, two patients underwent splenectomy to increase platelet count prior to re-treatment, 25 completed re-treatment of PEG IFN plus ribavirin combination therapy and 15 achieved SVR (genotype 1, $n = 11$; genotype 2, $n = 4$).

All of the patients who completed previous treatment also completed re-treatment and the baseline characteristics of those patients are shown in Table 1. Of the 86 genotype 1 patients, 54 were relapsers and 32 had shown NR to previous treatment. Of the 27 patients with genotype 2, 25 were relapsers and two had shown NR to previous treatment. Thirty-seven patients with genotype 1 and 14 patients with genotype 2 were assessed as IL-28B genotype, and 27 patients with genotype 1 and 10 patients with genotype 2 were assessed as ITPA genotype. There was no significant difference in the baseline characteristics between the previous treatment and the re-treatment with respect to peripheral blood cell counts, amino transaminase level and serum HCV RNA at the start of treatment (Table 1).

The baseline characteristics of patients with genotype 1 according to antiviral efficacy of the previous treatment are shown in Table 2. Among those with NR in the previous treatment, the rate of the minor allele of IL-28B was significantly higher than those with relapse in the previous treatment ($P < 0.01$). For genotype 1, the HCV RNA negative rate on re-treatment was 20% (17/86) at week 4, 61% (52/85) at week 12 and 76% (65/86) at week 24, and the SVR rate was 48% (41/86). The factors associated with SVR were assessed by univariate analysis and the factors of relapse after previous treatment and the serum HCV RNA level at the start of re-treatment were selected as being significant (Table 3). The SVR

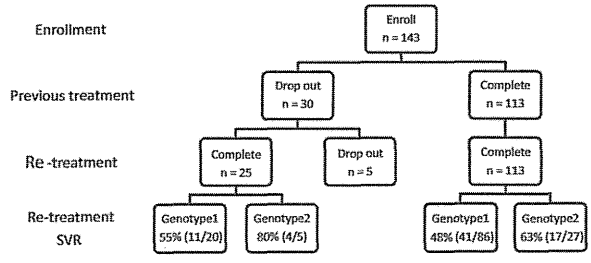

Figure 1 Patient flow for this study. SVR, sustained virological response.

Table 1 Baseline characteristics of patients and treatment factors in previous treatment and re-treatment

Factor	Genotype 1		Genotype 2	
No.	86		27	
Sex: male/female	46/40		15/12	
Effect of previous treatment: relapse/NR	54/32		25/2	
	Previous treatment	Re-treatment	Previous treatment	Re-treatment
PEG IFN type: α-2a/α-2b	0/86	41/45	0/27	6/21
Age (years)	58.1 ± 8.3	60.0 ± 8.5	58.9 ± 8.2	60.0 ± 8.1
White blood cells (/mm^3)	4779 ± 1383	4610 ± 1443	5195 ± 1473	4724 ± 1266
Neutrophils (/mm^3)	2478 ± 930	2355 ± 1071	2561 ± 827	2389 ± 941
Hemoglobin (g/dL)	13.7 ± 1.2	13.5 ± 1.7	14.4 ± 1.3	14.0 ± 1.2
Platelets (×10^4/mm^3)	16.0 ± 5.9	16.6 ± 6.2	18.0 ± 5.7	16.8 ± 5.2
ALT (IU/L)	75 ± 51	73 ± 72	57 ± 46	42 ± 32
Histology: activity, 0–1/2–3	29/29		11/7	
Fibrosis, 0–2/3–4	45/14		17/1	
Serum HCV RNA (KIU/mL)	1600	850	1500	700
IL-28B SNP: rs8099917; TT/TG	26/11		10/4	
ITPA SNP: rs1127354; CC/CA	20/7		9/1	
Core 70: wild/mutant	11/11			
Core 91: wild/mutant	15/7			
ISDR: 0–1/≥2	15/1			

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism.

rates of relapsers were significantly higher than those of patients with NR in the previous treatment (relapse, 67%, 36/54 vs NR, 16%, 5/32, $P < 0.0001$). As for the serum HCV RNA level at the start of re-treatment, although the SVR rate of those patients with 5 $\log_{10}$ IU/mL or more of HCV RNA was 38% (26/69), all patients with less than 5 $\log_{10}$ IU/mL of HCV RNA attained SVR (11/11) ($P = 0.0001$). As for the IL-28B genotype, among the patients with the major allele, the p-EVR rate was significantly higher and the EOT response rate showed marginal significance compared to that with the minor allele (p-EVR rate, 100%, 23/23 vs 30%, 3/10, $P < 0.0001$, EOT rate, 92%, 24/26 vs 64%, 7/11, $P = 0.05$). There was no significant difference of the SVR rate between major and minor alleles (major, 65%, 17/26 vs minor, 36%, 4/11, $P = 0.15$).

Figure 2(a) shows the result of stratified analysis according to the previous treatment response and HCV RNA at the start of re-treatment. The significant difference in SVR observed between high (≥5 $\log_{10}$ IU/mL) and low (<5 $\log_{10}$ IU/mL) baseline viral loads was still found in both previous relapsers ($P = 0.02$) and previous non-responders ($P = 0.02$). In patients with a high baseline viral load, previous relapsers achieved a higher SVR rate than previous non-responders ($P < 0.0001$). Next, the results of stratified analyses according to IL-28B genotype and previous treatment response or HCV RNA at the start of re-treatment showed no significant difference in SVR rates between the IL-28B genotype in patients with relapse after previous treatment ($P = 0.63$) (Fig. 2b). All patients with less than 5 $\log_{10}$ IU/mL of HCV RNA achieved SVR despite their IL-28B genotype and the SVR rates of patients with 5 $\log_{10}$ IU/mL or more of HCV RNA did not differ between IL-28B genotypes (Fig. 2c). Multivariate analysis among the factors of relapse to previous treatment response, HCV RNA at the start of re-treatment and IL-28B genotype showed that relapse after previous treatment response bore the most predictable relationship to SVR in re-treatment ($P = 0.074$).

As for the efficacy of re-treatment according to treatment duration among patients with HCV RNA negativity during re-treatment, the SVR rate of 72-week treatment was significantly higher than that of 48-week treatment (72 weeks, 73%, 29/40, vs 48 weeks, 52%, 12/25, $P < 0.05$). This significant difference was especially found in patients who attained c-EVR but not RVR on re-treatment (72 weeks, 73%, 16/22, vs 48 weeks,

Table 2 Baseline characteristics of patients and treatment factors according to the virological response in previous treatment among patients with genotype 1

Factor	Relapser in previous treatment		NR in previous treatment	
No.	54		32	
Sex: male/female	28/26		18/14	
	Previous treatment	Re-treatment	Previous treatment	Re-treatment
PEG IFN type: α-2a/α-2b	0/54	29/25	0/32	12/20
Age (years)	58.1 ± 8.1	60.3 ± 8.4	57.9 ± 8.9	59.6 ± 8.8
White blood cells (/mm^3)	4917 ± 1290	4692 ± 1035	4546 ± 1520	4462 ± 1993
Neutrophils (/mm^3)	2618 ± 846	2479 ± 805	2225 ± 1033	2105 ± 1454
Hemoglobin (g/dL)	13.9 ± 1.2	13.7 ± 1.6	13.5 ± 1.3	13.1 ± 1.9
Platelets (×10^4/mm^3)	17.1 ± 6.3	17.7 ± 6.1	14.1 ± 4.7	14.7 ± 6.2
ALT (IU/L)	75 ± 57	70 ± 76	75 ± 39	78 ± 64
Histology: activity, 0–1/2–3	20/18		9/11	
Fibrosis, 0–2/3–4	31/8		14/6	
Serum HCV RNA (KIU/mL)	1600	980	1550	800
IL-28B SNP: rs8099917; TT/TG	24/5		2/6	
ITPA SNP: rs1127354; CC/CA	15/6		5/1	
Core 70: wild/mutant	6/6		5/5	
Core 91: wild/mutant	9/3		6/4	
ISDR: 0–1/≥2	9/0		6/1	

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism.

38%, 5/13, $P < 0.05$) but not in patients who attained RVR or LVR (Fig. 3).

In genotype 2, the HCV RNA negative rate on re-treatment was 59% (16/27) at week 4, 85% (23/27) at week 12 and 93% (25/27) at week 24, and the SVR rate was 63% (17/27). The two patients with NR in previous treatment did not attain SVR with re-treatment. The factors associated with SVR were assessed by univariate analysis and only the factor of younger age at the start of re-treatment showed marginal significance ($P = 0.06$) (Table 4). Among the patients with RVR on re-treatment, the SVR rates were similar at 75% (6/8) to those with 24-week and 48-week treatment.

DISCUSSION

PAST STUDIES HAVE revealed that the factors of age, sex, progression of liver fibrosis, value of HCV RNA, number of mutations in the ISDR, amino acid substitutions in the core region, drug adherence and treatment duration show association with HCV eradication in PEG IFN plus ribavirin combination for naïve patients with CH-C.[3–5,25–33] Recently, the IL-28B genotype has been reported to be the most powerful factor associated with the antiviral effect of this combination therapy.[21–25] While the predictive factors for SVR in PEG IFN plus ribavirin combination therapy for naïve patients have been actively analyzed, those factors for patients who had already experienced this therapy are still unclear. Especially needing assessment is the correlation between IL-28B SNP or the previous treatment response and the antiviral effect in re-treatment. In this study, we tried to determine which factors could most effectively predict the antiviral effect in re-treatment.

In the present study, patients with relapse after the previous treatment and patients with a low serum HCV RNA level at the start of re-treatment showed significantly different results in this study of re-treatment of CH-C patients who had previously failed to attain SVR with PEG IFN plus ribavirin therapy. This result was similar to those of the EPIC[3] study on relapse and NR[17] and the SYREN trial of NR.[18] On the other hand, there was no significant difference between the influence of the IL-28B genotype and SVR. More specifically, if the previous treatment response was the same, there was no difference regardless of the IL-28B genotype. Considering this result, in re-treatment, the previous treatment response was a more effective predictive factor than IL-28B genotype. However, further investigation is needed to clarify the association between IL-28B

Table 3 Factors associated with a sustained virological response in re-treatment with PEG IFN plus ribavirin in patients with genotype 1

Factor		SVR	Non-SVR	*P*-value
No. of patients		41	45	
Age (years)		60.2 ± 7.1	59.9 ± 9.6	0.71
Sex: male/female		24/17	22/23	0.40
Serum HCV RNA (log IU/mL)		5.8 ± 1.4	6.4 ± 0.6	0.11
Serum HCV RNA: <5 log/≥5 log		11/28	0/43	<0.001
White blood cells (/mm^3)		4656 ± 1029	4566 ± 1763	0.42
Neutrophils (/mm^3)		2443 ± 804	2259 ± 1301	0.16
Hemoglobin (g/dL)		13.5 ± 1.6	13.4 ± 1.8	0.80
Platelets (×10^4/mm^3)		16.9 ± 5.7	16.3 ± 6.7	0.36
ALT (IU/L)		68 ± 69	78 ± 75	0.43
IL-28B SNP: TT/TG		17/4	9/7	0.15
ITPA SNP: CC/CA		13/3	7/4	0.39
Core 70: wild/mutant		5/4	6/7	1.00
Core 91: wild/mutant		7/3	8/5	1.00
ISDR: 0–1/≥2		9/0	6/1	0.44
PEG IFN: α-2a/α-2b		16/25	25/20	0.14
PEG IFN dose (μg/kg per week)	α-2a	2.91 ± 0.77	2.74 ± 0.69	0.61
	α-2b	1.25 ± 0.39	1.20 ± 0.32	0.59
Ribavirin dose (mg/kg per day)		9.34 ± 2.72	9.64 ± 3.20	0.51
1st treatment virological response	Relapse/NR	36/5	18/27	<0.001

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism; SVR, sustained virological response.

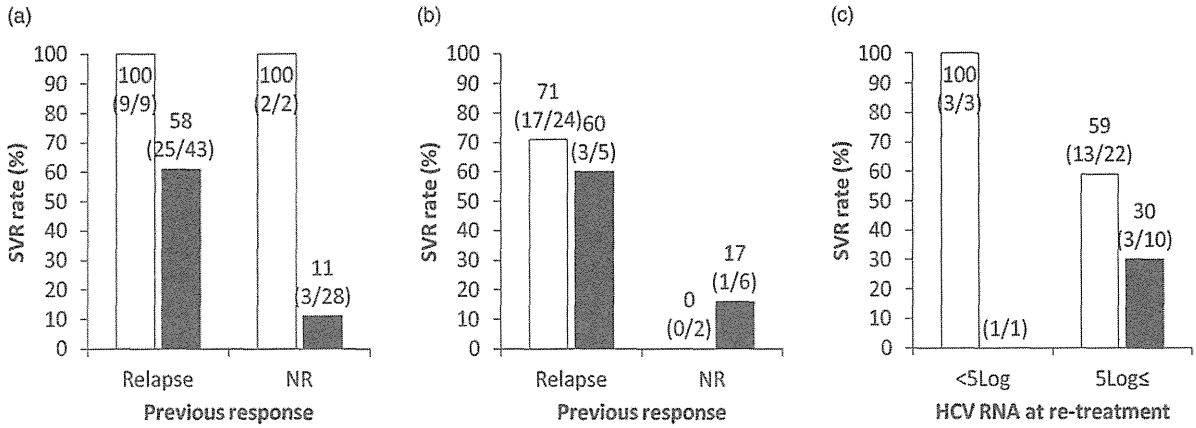

Figure 2 Sustained virological response (SVR) rates according to previous virological response, hepatitis C virus (HCV) RNA at start of re-treatment and genotype of interleukin (IL)-28B single nucleotide polymorphism (SNP) in patients with genotype 1. (a) Stratified analysis of previous virological response and HCV RNA at start of re-treatment. □, HCV RNA <5 log IU/mL at start of re-treatment; ■, HCV RNA ≥5 log IU/mL at start of re-treatment. (b) Stratified analysis of previous virological response and genotype of IL-28B SNP. □, Patients with major allele of IL-28B SNP; ■, patients with minor allele of IL-28B SNP. (c) Stratified analysis of HCV RNA at start of re-treatment and genotype of IL-28B SNP. □, Patients with major allele of IL28B SNP; ■, patients with minor allele of IL-28B SNP.