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.

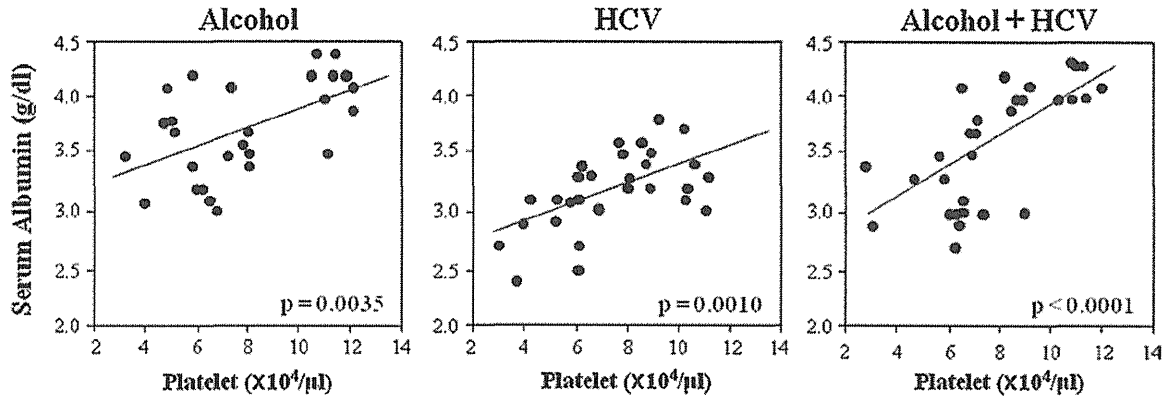

Figure 1. Correlation between serum albumin levels and platelet counts. Although the correlation was significant in each group, the levels of albumin in the alcohol group were always higher than in the HCV group. The approximate correlation line of the alcohol + HCV group was intermediate between the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

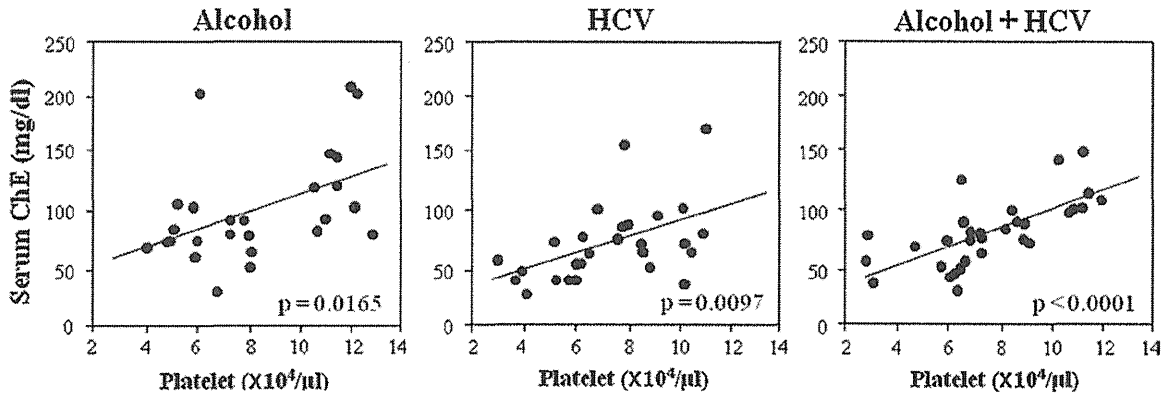

Figure 2. Correlation between serum cholinesterase levels and platelet counts. The correlations were almost similar in all three groups.

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.

References

1. Di Sario A, Feliciangeli G, Bendia E and Benedetti A: Diagnosis of liver fibrosis. Eur Rev Med Pharmacol Sci 8: 11-18, 2004.
2. Mullin EJ, Metcalfe MS and Maddern GJ: How much liver resection is too much? Am J Surg 190: 87-97, 2005.
3. Fazakas J, Mandli T, Ther G, *et al*: Evaluation of liver function for hepatic resection. Transplant Proc 38: 798-800, 2006.
4. Schneider PD: Preoperative assessment of liver function. Surg Clin North Am 84: 355-373, 2004.
5. Park DK, Um SH, Lee JW, *et al*: Clinical significance of variceal hemorrhage in recent years in patients with liver cirrhosis and esophageal varices. J Gastroenterol Hepatol 19: 1042-1051, 2004.
6. Forns X, Ampurdanès S, Llovet JM, *et al*: Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 36: 986-992, 2002.
7. Wai CT, Greenson JK, Fontana RJ, Kalbfieisch JD, Marrero JA, Conjeevaram HS and Lok AS: A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 38: 518-516, 2003.
8. Manguso F, D'Ambra G, Menchise A, Sollazzo R and D'Agostino L: Effects of an appropriate oral diet on the nutritional status of patients with HCV-related liver cirrhosis: a prospective study. Clin Nutr 24: 751-759, 2005.
9. Wadenvik H, Denfors I and Kutti J: Splenic blood flow and intrasplenic platelet kinetics in relation to spleen volume. Br J Haematol 67: 181-185, 1987.
10. Izuno K, Fujiyama S, Shibata J, Yoshida K, Sato T, Shimomura O and Takahashi M: Transrectal portal scintigraphy with I123 iodoamphetamine in liver diseases. Hepatogastroenterology 38: S8-S11, 1991.
11. Tatsumi C, Kudo M, Ueshima K, *et al*: Noninvasive evaluation of hepatic fibrosis using serum fibrotic markers, transient elastography (FibroScan) and real-time tissue elastography. Intervirology 51: 27-33, 2008.
12. Poniachik J, Bernstein DE, Reddy KR, Jeffers LJ, Coelho-Little ME, Civantos F and Schiff ER: The role of laparoscopy in the diagnosis of cirrhosis. Gastrointest Endosc 43: 568-571, 1996.
13. Regev A, Berho M, Jeffers LJ, *et al*: Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 97: 2614-2618, 2002.
14. Fontana RJ, Goodman ZG, Dienstag JL, *et al*: Relationship of serum fibrosis markers with liver fibrosis stage and collagen content in patients with advanced chronic hepatitis C. Hepatology 47: 789-798, 2008.
15. Annoni G, Weiner FR, Colombo M, Czaja MJ and Zern MA: Albumin and collagen gene regulation in alcohol- and virus-induced human liver disease. Gastroenterology 98: 197-202, 1990.

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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

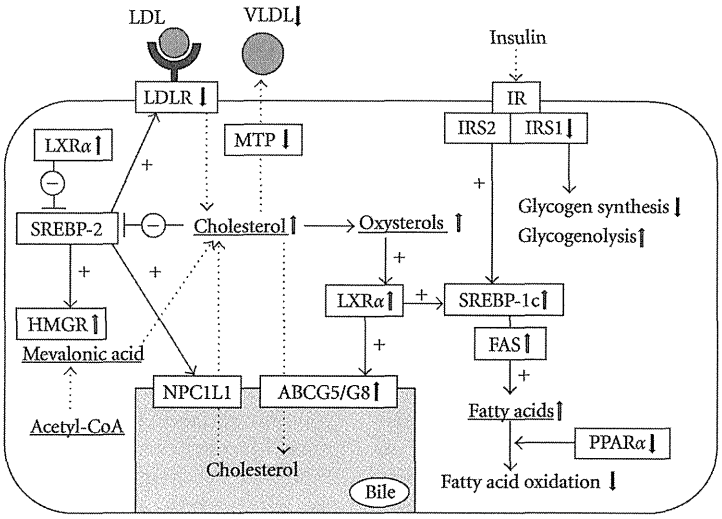

FIGURE 1: Expression profile of lipid metabolism-associated factors in chronic hepatitis C. ABCG5/G8, ATP-binding cassette G5/G8; FAS, fatty acid synthase; HMGR, HMG-CoA reductase; IR, insulin receptor; IRS, insulin receptor substrate; LDLR, LDL receptor; LXRα, liver X receptor α; MTP, microsomal triglyceride 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.

References

[1] P. J. Scheuer, P. Ashrafzadeh, S. Sherlock, D. Brown, and G. M. Dusheiko, "The pathology of hepatitis C," *Hepatology*, vol. 15, no. 4, pp. 567–571, 1992.

[2] S. J. Hwang and S. D. Lee, "Hepatic steatosis and hepatitis C: still unhappy bedfellows?" *Journal of Gastroenterology and Hepatology*, vol. 26, no. 1, pp. 96–101, 2011.

[3] A. Yamaguchi, S. Tazuma, T. Nishioka et al., "Hepatitis C virus core protein modulates fatty acid metabolism and thereby causes lipid accumulation in the liver," *Digestive Diseases and Sciences*, vol. 50, no. 7, pp. 1361–1371, 2005.

[4] V. Pazienza, S. Clément, P. Pugnale et al., "The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms," *Hepatology*, vol. 45, no. 5, pp. 1164–1171, 2007.

[5] J. M. Hui, A. Sud, G. C. Farrell et al., "Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression," *Gastroenterology*, vol. 125, no. 6, pp. 1695–1704, 2003.

[6] A. Lonardo, L. E. Adinolfi, P. Loria, N. Carulli, G. Ruggiero, and C. P. Day, "Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease," *Gastroenterology*, vol. 126, no. 2, pp. 586–597, 2004.

[7] E. E. Powell, J. R. Jonsson, and A. D. Clouston, "Steatosis: cofactor in other liver diseases," *Hepatology*, vol. 42, no. 1, pp. 5–13, 2005.

[8] M. E. Miquilena-Colina, E. Lima-Cabello, S. Sánchez-Campos et al., "Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in non-alcoholic steatohepatitis and chronic hepatitis C," *Gut*, vol. 60, no. 10, pp. 1394–1402, 2011.

[9] E. Bugianesi, F. Salamone, and F. Negro, "The interaction of metabolic factors with HCV infection: does it matter?" *Journal of Hepatology*, vol. 56, supplement 1, pp. S56–S65, 2012.

[10] M. Enjoji, K. Kotoh, M. Kato et al., "Therapeutic effect of ARBs on insulin resistance and liver injury in ptients with NAFLD and chronic hepatitis C: a pilot study," *International Journal of Molecular Medicine*, vol. 22, no. 4, pp. 521–527, 2008.

[11] R. Moucari, T. Asselah, D. Cazals-Hatem et al., "Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis," *Gastroenterology*, vol. 134, no. 2, pp. 416–423, 2008.

[12] R. Simó, A. Lecube, J. Genescà, J. I. Esteban, and C. Hernández, "Sustained virological response correlates with reduction in the incidence of glucose abnormalities in patients with chronic hepatitis C virus infection," *Diabetes Care*, vol. 29, no. 11, pp. 2462–2466, 2006.

[13] T. Kawaguchi, T. Ide, E. Taniguchi et al., "Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate 1 and 2," *American Journal of Gastroenterology*, vol. 102, no. 3, pp. 570–576, 2007.

[14] M. Alberstein, T. Zornitzki, Y. Zick, and H. Knobler, "Hepatitis C core protein impairs insulin downstream signalling and regulatory role of IGFBP-1 expression," *Journal of Viral Hepatitis*, vol. 19, no. 1, pp. 65–71, 2012.

[15] S. Pascarella, S. Clément, K. Guilloux, S. Conzelmann, F. Penin, and F. Negro, "Effects of hepatitis C virus on suppressor of cytokine signaling mRNA levels: comparison between different genotypes and core protein sequence analysis," *Journal of Medical Virology*, vol. 83, no. 6, pp. 1005–1015, 2011.

[16] K. Ueki, T. Kondo, and C. R. Kahn, "Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms," *Molecular and Cellular Biology*, vol. 24, no. 12, pp. 5434–5446, 2004.

[17] L. Rui, M. Yuan, D. Frantz, S. Shoelson, and M. F. White, "SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2," *Journal of Biological Chemistry*, vol. 277, no. 44, pp. 42394–42398, 2002.

[18] T. Kawaguchi, T. Yoshida, M. Harada et al., "Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3," *American Journal of Pathology*, vol. 165, no. 5, pp. 1499–1508, 2004.

[19] H. Knobler, T. Zhornicky, A. Sandler, N. Haran, Y. Ashur, and A. Schattner, "Tumor necrosis factor-α-induced insulin resistance may mediate the hepatitis C virus-diabetes association," *American Journal of Gastroenterology*, vol. 98, no. 12, pp. 2751–2756, 2003.

[20] S. Fernández-Veledo, R. Hernandez, T. Teruel, J. Mas, M. Ros, and M. Lorenzo, "Ceramide mediates TNF-α-induced insulin resistance on GLUT4 gene expression in brown adipocytes," *Archives of Physiology and Biochemistry*, vol. 112, no. 1, pp. 13–22, 2006.

[21] K. L. Donnelly, C. I. Smith, S. J. Schwarzenberg, J. Jessurun, M. D. Boldt, and E. J. Parks, "Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease," *Journal of Clinical Investigation*, vol. 115, no. 5, pp. 1343–1351, 2005.

[22] S. Dharancy, M. Lemoine, P. Mathurin, L. Serfaty, and L. Dubuquoy, "Peroxisome proliferator-activated receptors in HCV-related infection," *PPAR Research*, vol. 2009, Article ID 357204, 5 pages, 2009.

[23] S. McPherson, J. R. Jonsson, H. D. Barrie, P. O'Rourke, A. D. Clouston, and E. E. Powell, "Investigation of the role of SREBP-1c in the pathogenesis of HCV-related steatosis," *Journal of Hepatology*, vol. 49, no. 6, pp. 1046–1054, 2008.

[24] K. H. Kim, S. P. Hong, K. Kim, M. J. Park, K. J. Kim, and J. Cheong, "HCV core protein induces hepatic lipid accumulation by activating SREBP1 and PPARγ," *Biochemical and Biophysical Research Communications*, vol. 355, no. 4, pp. 883–888, 2007.

[25] G. Perlemuter, A. Sabile, P. Letteron et al., "Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis," *FASEB Journal*, vol. 16, no. 2, pp. 185–194, 2002.

[26] A. De Gottardi, V. Pazienza, P. Pugnale et al., "Peroxisome proliferator-activated receptor-α and -γ mRNA levels are reduced in chronic hepatitis C with steatosis and genotype 3 infection," *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 1, pp. 107–114, 2006.

[27] M. Nakamuta, R. Yada, T. Fujino et al., "Changes in the expression of cholesterol metabolism-associated genes in HCV-infected liver: a novel target for therapy?" *International Journal of Molecular Medicine*, vol. 24, no. 6, pp. 825–828, 2009.

[28] T. Fujino, M. Nakamuta, R. Yada et al., "Expression profile of lipid metabolism-associated genes in hepatitis C virus-infected human liver," *Hepatology Research*, vol. 40, no. 9, pp. 923–929, 2010.

[29] M. Nakamuta, T. Fujino, R. Yada et al., "Impact of cholesterol metabolism and the LXRα-SREBP-1c pathway on nonalcoholic fatty liver disease," *International Journal of Molecular Medicine*, vol. 23, no. 5, pp. 603–608, 2009.

[30] M. Enjoji, K. Yasutake, M. Kohjima, and M. Nakamuta, "Nutrition and nonalcoholic fatty liver disease: the significance of cholesterol," *International Journal of Hepatology*, vol. 2012, Article ID 925807, 6 pages, 2012.

[31] G. H. Syed, Y. Amako, and A. Siddiqui, "Hepatitis C virus hijacks host lipid metabolism," *Trends in Endocrinology and Metabolism*, vol. 21, no. 1, pp. 33–40, 2010.

[32] J. McLauchlan, "Lipid droplets and hepatitis C virus infection," *Biochimica et Biophysica Acta*, vol. 1791, no. 6, pp. 552–559, 2009.

[33] H. Tang and H. Grisé, "Cellular and molecular biology of HCV infection and hepatitis," *Clinical Science*, vol. 117, no. 2, pp. 49–65, 2009.

[34] M. Nakamuta, T. Fujino, R. Yada et al., "Expression profiles of genes associated with viral entry in HCV-infected human liver," *Journal of Medical Virology*, vol. 83, no. 5, pp. 921–927, 2011.

[35] M. Enjoji, M. Nakamuta, N. Kinukawa et al., "Beta-lipoproteins influence the serum level of hepatitis C virus," *Medical Science Monitor*, vol. 6, no. 5, pp. 841–844, 2000.

[36] S. Molina, V. Castet, C. Fournier-Wirth et al., "The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus," *Journal of Hepatology*, vol. 46, no. 3, pp. 411–419, 2007.

[37] S. B. Kapadia and F. V. Chisari, "Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 7, pp. 2561–2566, 2005.

[38] A. Parfieniuk and R. Flisiak, "Role of cannabinoids in chronic liver diseases," *World Journal of Gastroenterology*, vol. 14, no. 40, pp. 6109–6114, 2008.

[39] D. Osei-Hyiaman, M. DePetrillo, P. Pacher et al., "Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity," *Journal of Clinical Investigation*, vol. 115, no. 5, pp. 1298–1305, 2005.

[40] W. I. Jeong, D. Osei-Hyiaman, O. Park et al., "Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver," *Cell Metabolism*, vol. 7, no. 3, pp. 227–235, 2008.

[41] V. Purohit, R. Rapaka, and D. Shurtleff, "Role of cannabinoids in the development of fatty liver (steatosis)," *AAPS Journal*, vol. 12, no. 2, pp. 233–237, 2010.

[42] S. Lotersztajn, F. Teixeira-Clerc, B. Julien et al., "CB2 receptors as new therapeutic targets for liver diseases," *British Journal of Pharmacology*, vol. 153, no. 2, pp. 286–289, 2008.

[43] F. Teixeira-Clerc, M. P. Belot, S. Manin et al., "Beneficial paracrine effects of cannabinoid receptor 2 on liver injury and regeneration," *Hepatology*, vol. 52, no. 3, pp. 1046–1059, 2010.

[44] C. Hézode, E. S. Zafrani, F. Roudot-Thoraval et al., "Daily cannabis use: a novel risk factor of steatosis severity in patients with chronic hepatitis C," *Gastroenterology*, vol. 134, no. 2, pp. 432–439, 2008.

[45] A. Mallat, C. Hezode, and S. Lotersztajn, "Environmental factors as disease accelerators during chronic hepatitis C," *Journal of Hepatology*, vol. 48, no. 4, pp. 657–665, 2008.

[46] B. Julien, P. Grenard, F. Teixeira-Clerc et al., "Antifibrogenic role of the cannabinoid receptor CB2 in the liver," *Gastroenterology*, vol. 128, no. 3, pp. 742–755, 2005.

[47] F. Teixeira-Clerc, B. Julien, P. Grenard et al., "CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis," *Nature Medicine*, vol. 12, no. 6, pp. 671–676, 2006.

[48] A. Mallat and S. Lotersztajn, "Endocannabinoids and Liver Disease. I. Endocannabinoids and their receptors in the liver," *American Journal of Physiology*, vol. 294, no. 1, pp. G9–G12, 2007.

[49] S. Bátkai, Z. Járai, J. A. Wagner et al., "Endocannabinoids acting at vascular CB1 receptors mediate the vasodilated state in advanced liver cirrhosis," *Nature Medicine*, vol. 7, no. 7, pp. 827–832, 2001.

[50] C. M. Fernández-Rodriguez, J. Romero, T. J. Petros et al., "Circulating endogenous cannabinoid anandamide and portal, systemic and renal hemodynamics in cirrhosis," *Liver International*, vol. 24, no. 5, pp. 477–483, 2004.

[51] S. V. Siegmund, T. Qian, S. De Minicis et al., "The endocannabinoid 2-arachidonoyl glycerol induces death of hepatic stellate cells via mitochondrial reactive oxygen species," *FASEB Journal*, vol. 21, no. 11, pp. 2798–2806, 2007.

[52] P. Pacher, S. Bátkai, and G. Kunos, "The endocannabinoid system as an emerging target of pharmacotherapy," *Pharmacological Reviews*, vol. 58, no. 3, pp. 389–462, 2006.

[53] T. Poynard, V. Ratziu, J. McHutchison et al., "Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C," *Hepatology*, vol. 38, no. 1, pp. 75–85, 2003.

[54] H. M. Patton, K. Patel, C. Behling et al., "The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients," *Journal of Hepatology*, vol. 40, no. 3, pp. 484–490, 2004.

[55] M. Eslam, R. Aparcero, T. Kawaguchi et al., "Meta-analysis: insulin resistance and sustained virological response in hepatitis C," *Alimentary Pharmacology and Therapeutics*, vol. 34, no. 3, pp. 297–305, 2011.

[56] P. Deltenre, A. Louvet, M. Lemoine et al., "Impact of insulin resistance on sustained response in HCV patients treated with pegylated interferon and ribavirin: a meta-analysis," *Journal of Hepatology*, vol. 55, no. 6, pp. 1187–1194, 2011.

[57] L. E. Adinolfi, L. Restivo, R. Zampino, A. Lonardo, and P. Loria, "Metabolic alterations and chronic hepatitis C: treatment strategies," *Expert Opinion on Pharmacotherapy*, vol. 12, no. 14, pp. 2215–2234, 2011.

[58] M. Romero-Gómez, M. Diago, R. J. Andrade et al., "Treatment of insulin resistance with metformin in naïve genotype 1 chronic hepatitis C patients receiving peginterferon alfa-2a plus ribavirin," *Hepatology*, vol. 50, no. 6, pp. 1702–1708, 2009.

[59] K. Overbeck, D. Genné, A. Golay, and F. Negro, "Pioglitazone in chronic hepatitis C not responding to pegylated interferon-α and ribavirin," *Journal of Hepatology*, vol. 49, no. 2, pp. 295–298, 2008.

[60] S. A. Harrison, F. M. Hamzeh, J. Han, P. K. Pandya, M. Y. Sheikh, and J. M. Vierling, "Chronic hepatitis C genotype 1 patients with insulin resistance treated with pioglitazone and peginterferon alfa-2a plus ribavirin," *Hepatology*. In press.

[61] M. Khattab, M. Emad, A. Abdelaleem et al., "Pioglitazone improves virological response to peginterferon α-2b/ribavirin combination therapy in hepatitis C genotype 4 patients with insulin resistance," *Liver International*, vol. 30, no. 3, pp. 447–454, 2010.

[62] L. Serfaty, L. Fartoux, and R. Poupon, "Pioglitazone as adjuvant therapy in chronic hepatitis C: sequential rather than concomitant administration with pegylated interferon and ribavirin?" *Journal of Hepatology*, vol. 50, no. 6, pp. 1269–1271, 2009.

[63] M. Ikeda, K. I. Abe, M. Yamada, H. Dansako, K. Naka, and N. Kato, "Different anti-HCV profiles of statins and their potential for combination therapy with interferon," *Hepatology*, vol. 44, no. 1, pp. 117–125, 2006.

[64] C. Wang, M. Gale, B. C. Keller et al., "Identification of FBL2 as a geranylgeranylated cellular protein required for hepatitis C virus RNA replication," *Molecular Cell*, vol. 18, no. 4, pp. 425–434, 2005.

[65] S. A. Harrison, L. Rossaro, K. Q. Hu et al., "Serum cholesterol and statin use predict virological response to peginterferon

and ribavirin therapy," *Hepatology*, vol. 52, no. 3, pp. 864–874, 2010.

[66] G. A. Rao and P. K. Pandya, "Statin therapy improves sustained virologic response among diabetic patients with chronic hepatitis C," *Gastroenterology*, vol. 140, no. 1, pp. 144–152, 2011.

[67] H. Sezaki, F. Suzuki, N. Akuta et al., "An open pilot study exploring the efficacy of fluvastatin, pegylated interferon and ribavirin in patients with hepatitis C virus genotype 1b in high viral loads," *Intervirology*, vol. 52, no. 1, pp. 43–48, 2009.

[68] T. Bader, J. Fazili, M. Madhoun et al., "Fluvastatin inhibits hepatitis C replication in humans," *American Journal of Gastroenterology*, vol. 103, no. 6, pp. 1383–1389, 2008.

[69] M. Shimada, S. Yoshida, R. Masuzaki, and D. Schuppan, "Pitavastatin enhances antiviral efficacy of standard pegylated interferon plus ribavirin in patients with chronic hepatitis C: a prospective randomized pilot study," *Journal of Hepatology*, vol. 56, no. 1, pp. 299–300, 2012.

[70] G. Z. Leu, T. Y. Lin, and J. T. A. Hsu, "Anti-HCV activities of selective polyunsaturated fatty acids," *Biochemical and Biophysical Research Communications*, vol. 318, no. 1, pp. 275–280, 2004.

[71] H. Huang, Y. Chen, and J. Ye, "Inhibition of hepatitis C virus replication by peroxidation of arachidonate and restoration by vitamin E," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 47, pp. 18666–18670, 2007.

[72] N. Zaima, T. Sugawara, D. Goto, and T. Hirata, "Trans geometric isomers of EPA decrease LXRα-induced cellular triacylglycerol via suppression of SREBP-1c and PGC-1β," *Journal of Lipid Research*, vol. 47, no. 12, pp. 2712–2717, 2006.

[73] S. Takaki, Y. Kawakami, M. Imamura et al., "Eicosapentaenoic acid could permit maintenance of the original ribavirin dose in chronic hepatitis C virus patients during the first 12 weeks of combination therapy with pegylated interferon-α and ribavirin: a prospective randomized controlled trial," *Intervirology*, vol. 50, no. 6, pp. 439–446, 2008.

[74] J. Ye, C. Wang, R. Sumpter, M. S. Brown, J. L. Goldstein, and M. Gale, "Disruption of hepatitis C virus RNA replication through inhibition of host protein geranylgeranylation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15865–15870, 2003.

[75] M. Ikeda and N. Kato, "Life style-related diseases of the digestive system: Cell culture system for the screening of anti-hepatitis C virus (HCV) reagents: suppression of HCV replication by statins and synergistic action with interferon," *Journal of Pharmacological Sciences*, vol. 105, no. 2, pp. 145–150, 2007.

[76] V. Suppiah, M. Moldovan, G. Ahlenstiel et al., "IL28B is associated with response to chronic hepatitis C interferon-α and ribavirin therapy," *Nature Genetics*, vol. 41, no. 10, pp. 1100–1104, 2009.

[77] Y. Tanaka, N. Nishida, M. Sugiyama et al., "Genome-wide association of IL28B with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C," *Nature Genetics*, vol. 41, no. 10, pp. 1105–1109, 2009.

[78] D. Ge, J. Fellay, A. J. Thompson et al., "Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance," *Nature*, vol. 461, no. 7262, pp. 399–401, 2009.

[79] C. N. Hayes, M. Kobayashi, N. Akuta et al., "HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy," *Gut*, vol. 60, no. 2, pp. 261–267, 2011.

[80] N. Akuta, F. Suzuki, Y. Kawamura et al., "Prediction of response to pegylated interferon and ribavirin in hepatitis C by polymorphisms in the viral core protein and very early dynamics of viremia," *Intervirology*, vol. 50, no. 5, pp. 361–368, 2007.

[81] A. El-Shamy, S. R. Kim, Y. H. Ide et al., "Polymorphisms of hepatitis C virus non-structural protein 5A and core protein and clinical outcome of pegylated-interferon/ribavirin combination therapy," *Intervirology*, vol. 55, no. 1, pp. 1–11, 2012.

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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

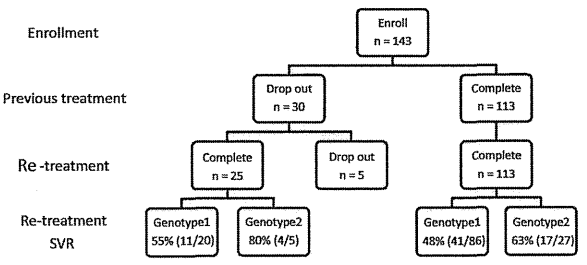

Figure 1 Patient flow for this study. SVR, sustained virological response.

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.

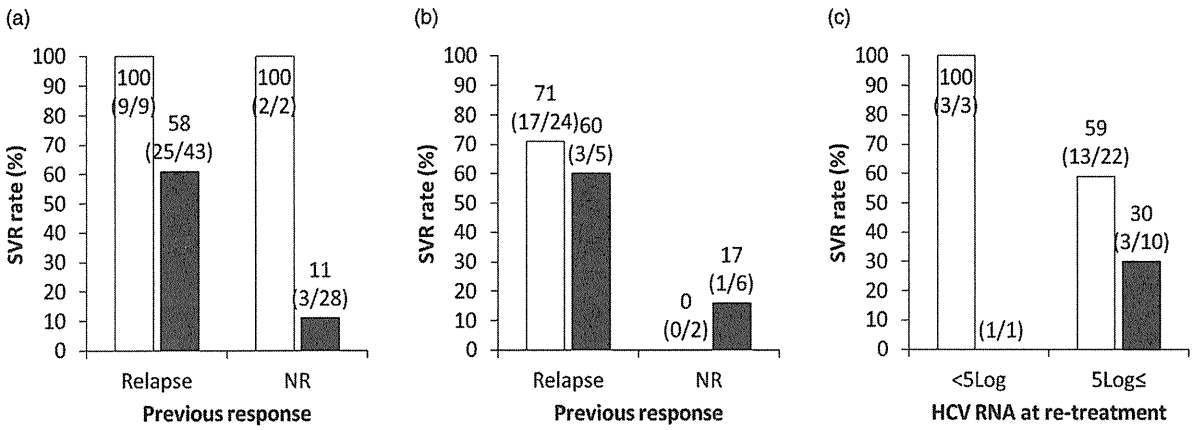

Figure 2 Sustained virological response (SVR) rates according to previous virological response, hepatitis C virus (HCV) RNA at start of re-treatment and genotype of interleukin (IL)-28B single nucleotide polymorphism (SNP) in patients with genotype 1. (a) Stratified analysis of previous virological response 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.

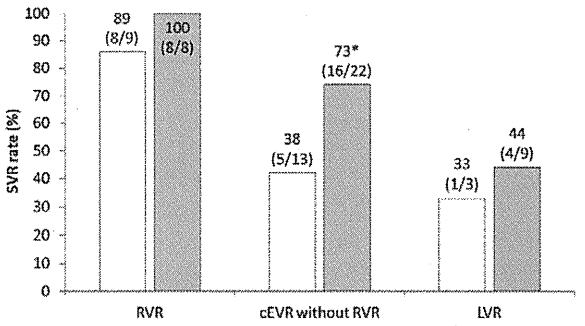

Figure 3 Sustained virological response (SVR) rates according to virological response in re-treatment and treatment duration in patients with genotype 1. □, Patients treated for 48 weeks; ▩, patients treated for 72 weeks. RVR, rapid virological response; cEVR, complete early virological response; LVR, late virological response. *$P < 0.05$; compared to 48 weeks of treatment.

genotype and antiviral effect of re-treatment because of their small number in this study. In this study, only one patient with the minor allele of IL-28B and NR in previous treatment could start and continue with the increased dose of PEG IFN (from 1.37 μg/kg in the previous treatment to 1.79 μg/kg in re-treatment) and ribavirin (from 10.3 mg/kg per day in the previous treatment to 11.1 mg/kg per day in re-treatment) and attained SVR by extended treatment. If the drug adherence does not improve, patients with the minor allele of IL-28B who show NR in the previous treatment should be treated with new drugs.

The next question is how the patients should be re-treated in order to attain SVR on re-treatment. In this study, the patients with a low serum HCV RNA level (<5 $\log_{10}$ IU/mL) at the start of re-treatment showed a significant rate of cure on re-treatment, and this is almost the same result as that previously reported.[16,17] In this study, the two patients with NR in the previous treatment and with less than 5 $\log_{10}$ IU/mL of HCV RNA level (20 KIU/mL and 52 KIU/mL of HCV RNA) at the start of re-treatment attained SVR. On the other hand, even if the previous treatment response was a relapse, the SVR rates were 58% (25/43) among the patients with 5 $\log_{10}$ IU/mL or more of HCV RNA. Because the HCV RNA level changed after the antiviral treatment, it is important to not miss the timing of when the HCV RNA level is low.

With respect to treatment duration among patients with HCV RNA negativiation during re-treatment, 72 weeks of treatment significantly increased the SVR rate compared to 48 weeks. This result was almost the same as that of the REPEAT study.[16] In our present study, the SVR rate among the patients with c-EVR but not RVR in re-treatment was significantly high by 72 weeks of treatment. On the other hand, the SVR rates among the

Table 4 Factors associated with a sustained virological response in re-treatment with PEG IFN plus ribavirin in patients with genotype 2

Factor		SVR	Non-SVR	*P*-value
No. of patients		17	10	
Age (years)		57.7 ± 8.8	63.7 ± 5.1	0.06
Sex: male/female		7/10	8/2	0.11
Serum HCV RNA (log IU/mL)		5.4 ± 1.4	6.1 ± 0.8	0.15
Serum HCV RNA: <5 log/≥5 log		5/11	1/9	0.35
White blood cells (/mm^3)		5049 ± 1355	4171 ± 910	0.10
Neutrophils (/mm^3)		2556 ± 1064	1999 ± 404	0.24
Hemoglobin (g/dL)		14.1 ± 1.3	13.8 ± 1.6	0.51
Platelets (×10^4/mm^3)		17.9 ± 5.4	14.8 ± 4.3	0.17
ALT (IU/L)		38 ± 19	48 ± 47	0.71
IL-28B SNP: TT/TG		6/2	4/2	1.00
ITPA SNP: CC/CA		5/1	4/0	1.00
PEG IFN: α-2a/α-2b		4/13	2/8	1.00
PEG IFN dose (μg/kg per week)	α-2a	3.23 ± 0.34	2.24 ± 2.25	1.00
	α-2b	1.32 ± 0.28	1.18 ± 0.23	0.21
Ribavirin dose (mg/kg per day)		10.4 ± 2.21	10.1 ± 1.31	0.44
1st treatment virological response	RVR/non-RVR	4/13	3/7	1.00

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

patients with RVR in re-treatment were similar between the patients with 48 weeks and 72 weeks of treatment. Thus, patients with c-EVR but not RVR in re-treatment should be re-treated for a longer period. In order to attain better SVR, extended treatment duration is generally recommended for patients with on-treatment LVR, whereas standard treatment duration is considered to be sufficient for patients with on-treatment c-EVR. However, the present study revealed that, even if patients achieved c-EVR on re-treatment, 72 weeks of treatment seems to be better than 48 weeks for treatment-experienced patients. The majority of naïve patients showing on-treatment c-EVR could eradicate HCV with 48 weeks of treatment while some could not. In a treatment-experienced setting, patients who are able to respond early but not eradicate HCV would be selected, and therefore extended treatment may be needed.

With genotype 2, the SVR rate was relatively high (63%). The patients who could not attain SVR in re-treatment (two patients) showed NR in the previous treatment. Thus, the patients with genotype 2 and showing NR in previous treatment seemed to be difficult to treat and could be treated with other drugs. Among the patients with RVR in re-treatment, the SVR rates were similar among those with RVR in re-treatment between 24 weeks and 48 weeks of treatment. The effectiveness of extended treatment for the patients with genotype 2 in re-treatment could not be demonstrated because of their small number in this study. Further investigation is needed to clarify this.

In conclusion, this study shows that the efficacy of re-treatment for genotype 1 patients who failed to show SVR to previous treatment with PEG IFN plus ribavirin could be predicted from the previous treatment response and a low HCV RNA level at the start of re-treatment. Re-treatment for 72 weeks led to clinical improvement for genotype 1 patients with c-EVR and without RVR on re-treatment.

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REFERENCES

1 Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335–74.
2 Hayashi N, Takehara T. Antiviral therapy for chronic hepatitis C: past, present, and future. *J Gastroenterol* 2006; **41**: 17–27.
3 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958–65.
4 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975–82.
5 Hadziyannis SJ, Sette H, Jr, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346–55.
6 Zeuzem S, Hultcrantz R, Bourliere M *et al.* Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. *J Hepatol* 2004; **40**: 993–9.
7 McHutchison JG, Everson GT, Gordon SC *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360**: 1827–38.
8 Hezode C, Forestier N, Dusheiko G *et al.* Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; **360**: 1839–50.
9 McHutchison JG, Manns MP, Muir AJ *et al.* Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010; **362**: 1292–303.
10 Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N. Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. *J Hepatol* 2012; **56**: 78–84.
11 Hayashi N, Okanoue T, Tsubouchi H, Toyota J, Chayama K, Kumada H. Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C. *J Viral Hepat* 2012; **19**: 134–42.
12 Reesink HW, Fanning GC, Farha KA *et al.* Rapid HCV-RNA decline with once daily TMC435: a phase I study in healthy volunteers and hepatitis C patients. *Gastroenterology* 2010; **138**: 913–21.
13 Lok AS, Gardiner DF, Lawitz E *et al.* Preliminary study of two antiviral agents for hepatitis C genotype 1. *N Engl J Med* 2012; **366**: 216–24.
14 Chayama K, Takahashi S, Toyota J *et al.* Dual therapy with the NS5A inhibitor BMS-790052 and the NS3 protease inhibitor BMS-650032 in HCV genotype 1b-infected null responders. *Hepatology* 2012; **55**: 742–8.
15 Bacon BR, Shiffman ML, Mendes F *et al.* Retreating chronic hepatitis C with daily interferon alfacon-1/ribavirin after nonresponse to pegylated interferon/ribavirin: DIRECT results. *Hepatology* 2009; **49**: 1838–46.
16 Jensen DM, Marcellin P, Freilich B *et al.* Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: a randomized trial. *Ann Intern Med* 2009; **150**: 528–40.

17 Poynard T, Colombo M, Bruix J *et al.* Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. *Gastroenterology* 2009; **136**: 1618–28.
18 Chevaliez S, Hezode C, Soulier A *et al.* High-dose pegylated interferon-alpha and ribavirin in nonresponder hepatitis C patients and relationship with IL-28B genotype (SYREN trial). *Gastroenterology* 2011; **141**: 119–27.
19 Berg C, Goncales FL, Jr, Bernstein DE *et al.* Re-treatment of chronic hepatitis C patients after relapse: efficacy of peginterferon-alpha-2a (40 kDa) and ribavirin. *J Viral Hepat* 2006; **13**: 435–40.
20 Oze T, Hiramatsu N, Yakushijin T *et al.* Efficacy of re-treatment with pegylated interferon plus ribavirin combination therapy for patients with chronic hepatitis C in Japan. *J Gastroenterol* 2011; **46**: 1031–7.
21 Thomas DL, Thio CL, Martin MP *et al.* Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798–801.
22 Suppiah V, Moldovan M, Ahlenstiel G *et al.* IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100–4.
23 Tanaka Y, Nishida N, Sugiyama M *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105–9.
24 Thompson AJ, Muir AJ, Sulkowski MS *et al.* Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in hepatitis C virus-1 patients. *Gastroenterology* 2010; **139**: 120–9.
25 Kurosaki M, Tanaka Y, Nishida N *et al.* Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in IL28B and viral factors. *J Hepatol* 2011; **54**: 439–48.
26 Akuta N, Suzuki F, Kawamura Y *et al.* Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007; **46**: 403–10.
27 Berg T, von Wagner M, Nasser S *et al.* Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. *Gastroenterology* 2006; **130**: 1086–97.
28 Mangia A, Minerva N, Bacca D *et al.* Individualized treatment duration for hepatitis C genotype 1 patients: a randomized controlled trial. *Hepatology* 2008; **47**: 43–50.
29 Oze T, Hiramatsu N, Yakushijin T *et al.* The efficacy of extended treatment with pegylated interferon plus ribavirin in patients with HCV genotype 1 and slow virologic response in Japan. *J Gastroenterol* 2011; **46**: 944–52.
30 Oze T, Hiramatsu N, Yakushijin T *et al.* Indications and limitations for aged patients with chronic hepatitis C in pegylated interferon alfa-2b plus ribavirin combination therapy. *J Hepatol* 2011; **54**: 604–11.
31 McHutchison JG, Manns M, Patel K *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061–9.
32 Oze T, Hiramatsu N, Yakushijin T *et al.* Pegylated interferon alpha-2b (Peg-IFN alpha-2b) affects early virologic response dose-dependently in patients with chronic hepatitis C genotype 1 during treatment with Peg-IFN alpha-2b plus ribavirin. *J Viral Hepat* 2009; **16**: 578–85.
33 Hiramatsu N, Oze T, Yakushijin T *et al.* Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. *J Viral Hepat* 2009; **16**: 586–94.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Association of enhanced activity of indoleamine 2,3-dioxygenase in dendritic cells with the induction of regulatory T cells in chronic hepatitis C infection

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Abstract

Background Altered functions of dendritic cells (DCs) and/or increases of regulatory T cells (Tregs) are involved in the pathogenesis of chronic hepatitis C virus (HCV) infection. A tryptophan-catabolizing enzyme, indoleamine 2,3-dioxygenase (IDO), is reported to be an inducer of immune tolerance. Our aim was to clarify whether or not IDO is activated in chronic hepatitis C patients and its role in immune responses.

Methods This study enrolled 176 patients with chronic HCV infection and 37 healthy volunteers. Serum kynurenine concentration was evaluated by high-performance liquid chromatography, and its correlation with clinical parameters was examined. Monocyte-derived DCs were prepared from the subjects and subsequently stimulated with a combination of lipopolysaccharide and interferon-gamma to induce functional IDO (defined as IDO-DCs). The phenotypes, kynurenine or cytokine production, and T-cell responses with IDO-DCs were compared between the patients and healthy volunteers.

Results The serum kynurenine level in the patients was significantly higher than that in the healthy volunteers, and the level of serum kynurenine was positively correlated with the histological activity or fibrosis score. IDO activity in IDO-DCs from the patients was significantly higher than that in IDO-DCs from the volunteers. Furthermore, IDO-DCs from the patients induced more Tregs in vitro compared with those from the volunteers, and the frequency of induced Tregs by IDO-DCs was decreased with an IDO-specific inhibitor.

Conclusions Systemic IDO activity is enhanced in chronic hepatitis C patients in correlation with the degree of liver inflammation and fibrosis. In response to inflammatory stimuli, DCs from the patients tend to induce Tregs, with some of this action being dependent on IDO.

Keywords Hepatitis C virus · Dendritic cell · Regulatory T cell · Indoleamine 2,3-dioxygenase

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Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide. It is estimated that 170 million people

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