

Amino Acid 70 Substitution in the Core Region of Hepatitis C Virus in Serum Lipid Markers of Patients with Chronic Hepatitis C Genotype 1b

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Abstract

Introduction: Previously, we reported that Hepatitis C Virus (HCV) infection had effects for lipid metabolism in patients with Chronic Hepatitis C (CHC). Recently, HCV with substitutions in amino acids (aa) 70 and/or 91 in the core region of HCV (HCV-C) has been reported to be more difficult to treat than HCV without aa 70 and 91 substitutions. The aim of this study was to clarify whether aa 70 or 91 substitution in the HCV-C influenced serum cholesterol fractions in patients with CHC genotype 1b and high viral load.

Patients and Methods: Twenty-two patients infected with genotype 1b and high viral loads, whose serum samples taken before the start of therapy had been stored at -80°C, and in whom aa 70 and 91 substitutions in the HCV-C could be detected, were selected. Patients without aa 70 and 91 substitutions in the HCV-C were assigned to wild (n=12), those with aa 70 substitution in the HCV-C assigned to mutant-70 (n=6), and those with aa 91 substitution in the HCV-C were assigned to mutant-91 (n=4). All patients received interferon (IFN)-based therapy. Fasting serum total cholesterol (C) and its fractions were compared before starting IFN therapy and at 24 weeks after the End of Therapy (EOT). When serum HCV-RNA was negative at 24 weeks after EOT, the patient was defined as having SVR. RESULTS: The SVR rates were 42% (5/12) in the wild, and 17% (1/6) in the mutant-70, and 0% (0/4) in the mutant-91. Serum levels of LDL-C were significantly lower and those of HDL-C were significantly higher in the mutant-70 patients than in the wild patients before starting therapy. Only serum level of VLDL-C increased significantly at 24 weeks after EOT than before starting therapy in the wild patients.

Conclusions: It was clarified that the mutant-70 influenced serum cholesterol fractions before starting therapy.

Keywords: Chronic hepatitis C; Amino acid substitution; Core region; Cholesterol; Fraction

Introduction

Hepatic biosynthesis of lipids has been suggested to play a role in the processes of infection and replication of Hepatitis C Virus (HCV) [1,2]. Serum cholesterol levels have been reported to be a predictor of Sustained Virologic Response (SVR) in patients with Chronic Hepatitis C (CHC) receiving antiviral therapy, but the precise mechanisms responsible for this phenomenon are uncertain [3-6]. We also reported that serum cholesterol levels increase in CHC patients showing SVR after treatment with interferon (IFN) based therapy and Corey et al. [9] reported similar observations [7-9].

HCV with amino acid (aa) 70 and/or 91 substitutions in the core region has been reported to be more difficult to treat than HCV without aa 70 and 91 substitutions [10,11]. However, the effects of aa 70 and/or 91 substitutions in the core region of HCV (HCV-C) on lipid metabolism have not been elucidated.

The goal of the present retrospective study was to clarify effects of aa 70 or 91 substitutions in HCV-C on serum Total Cholesterol (T-C) and its fractions in patients with CHC infected with genotype 1b and having high viral load.

Patients and Methods

Patients

Of 43 subjects in a previous study, 22 consecutive patients infected with genotype 1b (male/female: 10/12; age: 41 to 67 years; baseline HCV-RNA: 170 to >850 KIU/ml), whose serum samples taken immediately before the start of therapy had been stored at -80°C, and in whom aa 70 and 91 substitutions could be detected, were selected [7,8].

Informed consent for participation in this study was obtained from each patient.

All patients were positive for anti-HCV antibody (HCV-Ab) on testing with a third-generation Enzyme-Linked Immunosorbent Assay (ELISA) and were positive for serum HCV-RNA quantified by RT-PCR (original Amplicor Monitor Version 2.0 test; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), but were negative for Hepatitis B Surface Antigen (HBs-Ag). Patients were excluded if they were known to be homosexual or were intravenous drug users, as well as if they were positive for antinuclear antibodies, or if they had metabolic liver dysfunction, history of familial hyperlipidemia, HIV co-infection or renal dysfunction. Furthermore, patients were excluded if they were taking lipid-lowering drugs, if they were taking hypoglycemic agents, or if they had a history of habitual alcohol abuse

(daily alcohol consumption >20 g/day in men and >10 g/day in women) [9-11].

All patients underwent percutaneous liver biopsy under ultrasound guidance before treatment. Biopsy specimens were scored according to the Histology Activity Index (HAI) of Knodell et al. [12], and were divided into three grades (Grade 1, HAI score of 1-3; Grade 2, HAI score of 4-8; Grade 3, HAI score of 9 or more) [13]. Biopsy specimens were also divided into four stages (1-4) based on the fibrosis scores, as reported by Desmet [13].

Assessment of steatosis was performed as described previously [14]. Briefly, steatosis was evaluated by examining a minimum of 200 hepatocytes per hematoxylin eosin-stained section and counting the number of cells with microvesicular or macrovesicular changes, and this was then expressed as the percentage of liver cells containing fat. Subsequently, steatosis was graded as absent if <5% of the hepatocytes contained fat droplets, as mild if 5-20% contained fat, and as severe if >20% contained fat.

IFN regimens

After providing informed consent, patients with CHC were treated with IFN-alpha, or Pegylated (PEG) IFN-alpha 2b combined with ribavirin between February 2002 and May 2005 at our hospital. Eighteen patients were given a median dose of 6 (range: 6-9) MU of IFN-alpha 2b (Intron A; MSD. K.K, Tokyo, Japan) for a median of 38 (24-78) weeks, combined with a median dose of 600 (400-800) mg/day ribavirin (MSD. K.K) for a median of 24 (24-48) weeks. IFN-alpha 2b was administered daily for 2 weeks, followed by the same dose thrice weekly. Four patients were given a median dose of 100 (70-100) µg of PEG-IFN-alpha 2b (Pegintron; MSD. K.K) combined with a median dose of 600 (400-800) mg/day ribavirin for a median of 48 (32-48) weeks. PEG-IFN was administered weekly. Some patients needed slight adjustment of IFN and ribavirin doses due to decreases in leukocyte count, platelet count or hemoglobin levels during therapy.

Qualitative Amplicor Monitor Version 2.0 test (Chugai Pharmaceutical Co., Ltd.) for serum HCV was performed several times during and after treatment. When RT-PCR was negative at 24 weeks

after the end of therapy (EOT), this was defined as SVR, while a positive result at this time was defined as Non-Response (NVR) or relapse if undetectable HCV-RNA levels had been reached before EOT.

Assay

Fasting serum T-C and its fractions (high-density lipoprotein cholesterol: HDL-C; low-density lipoprotein cholesterol: LDL-C; and very low-density lipoprotein cholesterol: VLDL-C) were evaluated before starting IFN therapy and at 24 weeks after EOT [15]. Serum albumin and peripheral blood prothrombin time (%) were measured by standard methods before starting IFN therapy and at least 24 weeks after EOT.

Detection of aa 70 and 91 substitutions in the HCV-C was performed as reported previously, and the patients were divided into 3 groups based on the findings [11]. Patients without aa 70 (arginine) and 91 (leucine) substitutions in HCV-C were assigned to the wild-type group, patients with aa 70 substitution (glutamine/histidine) were assigned to the mutant-70-type group, and patients with aa 91 (methionine) substitution were assigned to the mutant-91-type group.

Body mass index (BMI) was calculated as body weight in kilograms divided by the square of body height in meters before treatment, at EOT, and at 24 weeks after EOT.

We compared the rates of SVR between patients in the wild-type and mutant-70-type groups, because the number of patients with mutant-91 was insufficient for significant differences to be examined. Furthermore, we compared serum levels of T-C and its fractions, including LDL-C, HDL-C and VLDL-C before starting therapy and at 24 weeks after EOT, liver tests before starting therapy, and BMI before starting therapy, at EOT, and at 24 weeks after EOT between the patients in the wild-type and mutant-70-type groups.

Statistical analysis

Results shown in the Table 1 are presented as medians and the 10th-90th percentiles.

			Non-Wild			
			Wild	Mutant-70	Mutant-91	
No. of patients			12	6	4	
Age	(years)	Median	61(45-65)	63(57-64)	60(47-64)	NS#
Sex	(M/F)		5/7	3/3	2/2	NS*
Serum HCV level	(KIU/ml)		535(354-742)	540(294-842)	670(338-757)	NS#
Pathological findings						
Activity (1/2/3)			(3 / 8 / 1)	(2 / 3 / 1)	(1 / 2 / 1)	NS*
Staging (1/2/3/4)			(4 / 3 / 5 / 0)	(1 / 3 / 0 / 2)	(0 / 2 / 2 / 0)	NS*
Fat accumulation (-)/(+)/(++)			(10/2/0)	(2/2/2)	(3/1/0)	P>0.05*
IFN-based therapy						
Kind of IFN						
IFN-alpha 2b	(n=)		10	4	4	

Period	(weeks)	36 (24-52)	24 (24-24)	32 (24-62)	NS#
Peg- IFN-alpha 2b	(n=)	2	2	0	
Period	(weeks)	48 (48-48)	36 (26-46)		NS#
Ribavirin	(n=)	12	6	4	
Period	(weeks)	36 (24-48)	24 (24-36)	24 (24-24)	NS#
Adherence					
IFN	(%)				
Ribavirin	(%)				
Viral kinetics					
(SVR/ Relapser / NVR)		(5 / 4 / 3)	(1 / 2 / 3)	(0 / 2 / 2)	

Table 1: Clinical background of patients with chronic hepatitis C, in parenthesis, range represents 10th to 90th percentiles.

Comparisons between 2 groups were performed by nonparametric Mann-Whitney test, Wilcoxon signed rank test, or chi-squared test. Multiple comparisons were performed by parametric Tukey's test. In all analyses, a probability value of less than 0.05 was considered to indicate statistical significance.

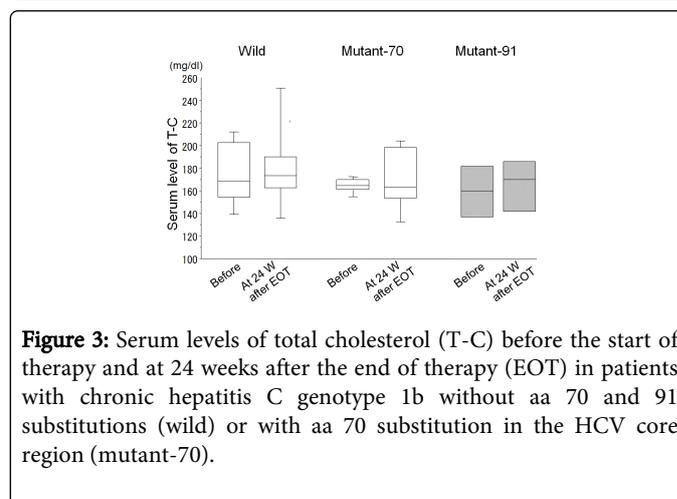
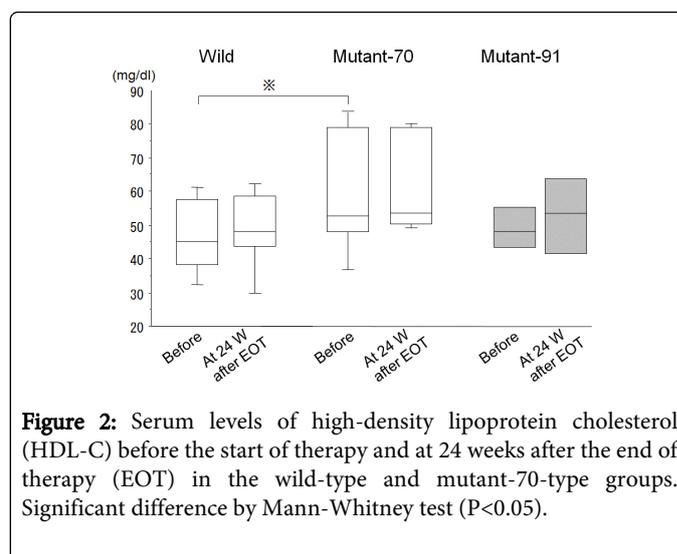
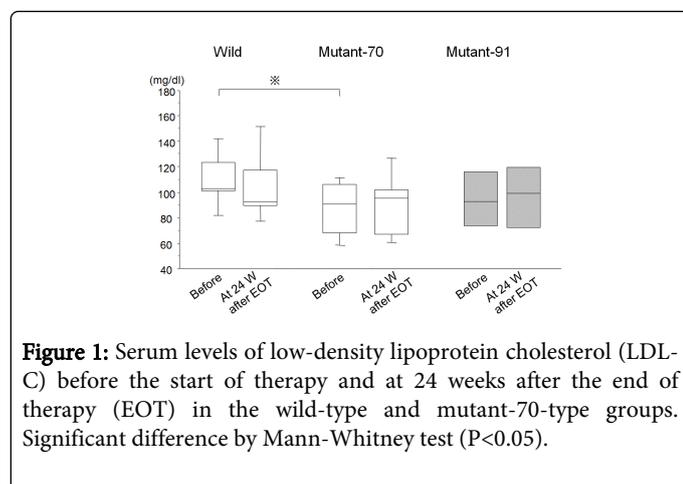
Results

Response of CHC to treatment

IFN-alpha or PEG-IFN-alpha 2b combined with ribavirin achieved SVR in 6 patients (5 in wild-type group, and 1 in mutant-70-type group) and NVR in 16 patients, including 8 primary NVRs (3 in wild-type group, 3 in mutant-70-type group, and 2 in mutant-91-type group) and 8 relapsers (4 in wild-type group, 2 in mutant-70-type group, and 2 in mutant-91-type group).

Changes in serum lipids in CHC patients

Serum levels of LDL-C were significantly higher ($P < 0.05$, Mann-Whitney test), and those of HDL-C were significantly lower in the wild-type group than in the mutant-70-type group before the start of therapy (Figures 1 and 2).



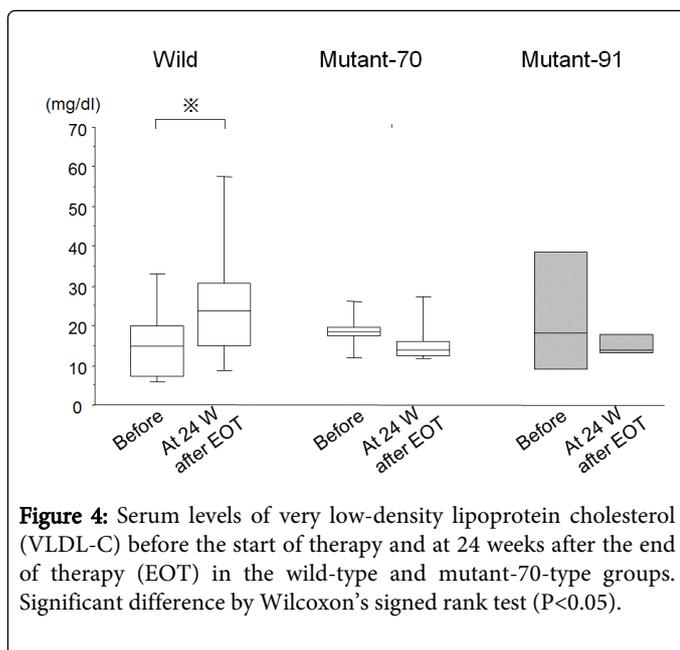


Figure 4: Serum levels of very low-density lipoprotein cholesterol (VLDL-C) before the start of therapy and at 24 weeks after the end of therapy (EOT) in the wild-type and mutant-70-type groups. Significant difference by Wilcoxon's signed rank test ($P < 0.05$).

Serum levels of VLDL-C were significantly higher ($P < 0.05$, Mann-Whitney test) at 24 weeks after EOT than before the start of therapy in the wild-type group (Figure 4), but T-C and its fractions did not significantly vary in the mutant-70-type group (Figure 1-4).

Changes in other serum liver markers in CHC patients

Serum albumin had a median level of 4.3 g/dl (10th-90th range: 3.7-4.7 g/dl) before the start of therapy, and a median of 4.6 g/dl (10th-90th range: 4.1-4.8 g/dl) at 24 weeks after EOT in the wild-type group, while albumin had a median level of 4.3 g/dl (10th-90th range: 4.0-4.5 g/dl) before the start of therapy, and a median of 4.4 g/dl (10th-90th range: 4.0-4.7 g/dl) at 24 weeks after EOT in the mutant-70-type group.

Peripheral blood prothrombin time was a median 94% (10th-90th range: 83-113%) before the start of therapy, and a median 97% (10th-90th range: 91-106%) at 24 weeks after EOT in the wild-type group, while prothrombin time was a median 96% (10th-90th range: 86-104%) before the start of therapy and a median 97% (10th-90th range: 83-115%) at 24 weeks after EOT in the mutant-70-type group.

There were no significant differences in serum levels of albumin and peripheral blood prothrombin time in either group before the start of therapy and at 24 weeks after EOT.

BMI during experimental period

BMI in the 3 groups tended to be lower at EOT, but there were no significant differences in BMI from before the start of therapy to 24 weeks after EOT in any of the 3 groups (Figure 5).

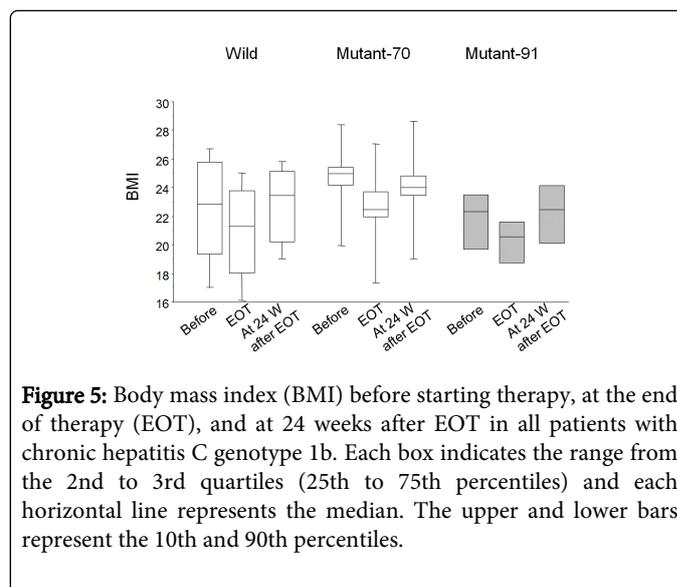


Figure 5: Body mass index (BMI) before starting therapy, at the end of therapy (EOT), and at 24 weeks after EOT in all patients with chronic hepatitis C genotype 1b. Each box indicates the range from the 2nd to 3rd quartiles (25th to 75th percentiles) and each horizontal line represents the median. The upper and lower bars represent the 10th and 90th percentiles.

Discussion

The present study demonstrated that patients in the mutant-70-type group had significantly lower serum LDL-C and higher serum HDL-C than patients in the wild-type group before the start of therapy. A case-control analysis [9] showed that patients with HCV infection had significantly lower serum cholesterol and LDL-C than controls (not infected with HCV), and we reported that serum levels of T-C were significantly lower in patients with CHC than in patients with chronic hepatitis B [7,8]. Similar results have been reported; HCV-antibody positivity was found to be independently associated with lower cholesterol and LDL-C levels, but not with lower levels of HDL-C or triglycerides, in HIV-HCV co-infected patients [16]. Therefore, chronic HCV infection indicates lower serum T-C and LDL-C. From the results of the present study, HCV genotype 1b, particularly mutant-70-type, appears to reduce serum LDL-C more strongly than wild-type HCV genotype 1b. The finding that higher baseline serum cholesterol and LDL-C is a predictor for SVR treated with PEG-IFN and ribavirin [3-6], may be explained by the fact that the majority of subjects within the study groups had wild-type HCV-C. The effects of mutant-91 could not be clarified, as the number of patients with mutant-91 was too small. Further studies including a larger number of patients are necessary in order to explore the relationship between serum LDL-C levels and aa 70 and/or 91 substitutions in HCV-C. The present study also clarified that serum VLDL-C is higher at 24 weeks after EOT than before the start of therapy, and this may reflect the number of SVR patients in the wild-type group. It is possible that mutant-70 lowers serum VLDL-C more strongly than the wild-type HCV genotype 1b, although the present study did not show any differences in serum levels of VLDL-C between the wild-type and mutant-70-type groups before the start of therapy.

Although hepatic steatosis did not differ in the 3 groups of patients infected genotype 1b, an inverse correlation has been reported to exist between serum cholesterol levels and hepatic steatosis, which is reversible with antiviral treatment in patients infected with HCV genotype 3 [17].

LDL-C and VLDL-C are Apo-B-containing lipoproteins. VLDL-C is generally secreted into the bloodstream after assembly with Apo-B.

If mutant-70 HCV 1b more strongly inhibits assembly and/or secretion of VLDL than wild-type HCV 1b, as is the case in acquired Hypobetalipoproteinemia (HBL) and Familial HBL (FHBL), the increased VLDL-C seen after SVR in the wild-type can be explained. The precise mechanism through which HCV induces acquired HBL remains unknown, but the interactions between HCV, apo-B and microsomal triglyceride transfer protein have been clarified [17].

On the other hand, serum levels of HDL-C were significantly higher in the mutant-70-type group than in the wild-type group before the start of therapy. This suggests that HDL-C is produced to greater degree by extrahepatic tissues or organs such as small intestines in order to supplement the decrease in serum LDL-C.

When cholesterol levels are discussed, the relationship with liver function becomes a problem, as serum cholesterol levels tend to decrease in patients who show histological progression of chronic liver disease [18]. In the present study, serum albumin and prothrombin time were measured in order to assess hepatic synthetic function, but there were no significant differences in these parameters between baseline and assessment. Therefore, the change in serum lipid markers does not necessarily depend on amelioration of liver function brought by eradication of HCV. Furthermore, interpretation of the lipid profile data should consider several factors that may bias comparisons between populations, such as obesity or dietary habits. Accordingly, we excluded patients with a history of alcohol abuse, as well as those receiving lipid-lowering drugs.

There were no significant differences in BMI from the start of therapy to 24 weeks after EOT, although BMI tended to be lower at EOT in all 3 groups, which may reflect the fact that ribavirin plus IFN-alpha2b and PEG-IFN-alpha 2b treatments are associated with decreased energy intake and weight loss [19,20].

There are several limitations in this study. First, the number of patients was small, and there may have been selection bias from the previous study. Second, we did not examine serum levels of cholesterol in CHC patients infected with other genotypes, and the IFN-based regimens differed. Therefore, a prospective study including a larger number of patients treated with the same regimen and patients infected with other genotypes is necessary. Recently, cell culture systems expressing HCV core protein of HCV genotypes 1 to 7 are developing with the advent of infectious cell culture systems [21]. So far, it is not clear difference influencing fat metabolism between the wild type and mutant-70-type in HCV-C, which may become apparent using a cultured cell infected with HCV in the near future.

In conclusion, our results suggest that chronic HCV infection with mutant-70-type genotype 1b reduces serum LDL-C and VLDL more than infection with wild-type HCV.

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