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

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Key Words

Hepatitis, chronic • Virus, hepatitis C • Statin • Interferon, pegylated • Ribavirin

Abstract

Objective: Response to pegylated (PEG) interferon (IFN) and ribavirin is achieved only in 40–50% of patients infected with hepatitis C virus (HCV) of genotype 1 in high viral loads, which needs to be improved. **Methods:** In an open-label pilot study, fluvastatin (HMG-CoA reductase inhibitor), 20 mg daily, was given along with PEG-IFN/ribavirin to 21 patients with chronic hepatitis C. They were followed for HCV RNA in serum. **Results:** During treatment for 48 weeks, HCV RNA was lost from serum in 93% of the patients. In the 15 patients who received 48-week therapy, a sustained virological response (SVR) with loss of HCV RNA 24 weeks after completion was achieved in 10 (67%), including 7 of the 9 (78%) male and 3 of the 6 (50%) female patients. In the remaining 6 patients who received 72-week therapy, SVR was gained in 4 (67%), including 1 of the 2 male and 3 of the 4 female patients aged 56, 58 and 62 years, respectively. **Conclusion:** Fluvastatin could be used safely to increase the response to PEG-IFN and ribavirin, especially in aged women who respond poorly to combined PEG-IFN/ribavirin.

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Introduction

Over the world approximately 190 million people are persistently infected with hepatitis C virus (HCV) [1], and about 30% of them develop serious liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC) [2]. Interferon (IFN) is the only drug that can resolve HCV infection. However, using the most advanced treatment currently available, with pegylated (PEG)-IFN and ribavirin for 24–48 weeks, a sustained virological response (SVR) defined by the loss of HCV RNA from serum 24 weeks after completion of therapy is achieved in 40–80% of the patients [3–5]. The response rate is influenced by host factors such as sex, age and ethnicity [6–8], as well as virological factors including genotypes and viral loads [9]. It remains unsatisfactorily low in patients infected with HCV genotype 1 at merely 40–50%, and dismal in women aged 50 years or older at merely 22% [10]. Hence, there is an imminent need to improve the efficacy of antiviral treatment for terminating HCV infection in these patients.

Efforts have been devoted toward increasing the efficacy to PEG-IFN/ribavirin therapy. Inhibitors of viral protease, alone or in combination with the IFN-based treatment, have been found effective in preliminary studies [11, 12]. Recently, drugs that can inhibit the key enzyme

for controlling the synthesis of cholesterol, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, have gained attention due to their potential to decrease the replication of HCV in clinical and experimental settings [13, 14].

More than three quarters of Japanese patients are infected with HCV genotype 1b in high viral loads. They are much older than Western patients owing to the widespread HCV infection which struck Japan about 20 years ago [15]. Owing to this, the response to PEG-IFN/ribavirin in Japanese patients with chronic hepatitis C is poorer than that in Western countries. In an open-label pilot study, 21 patients with chronic hepatitis C, who were infected with HCV-1b at high viral loads, received triple treatment with fluvastatin, PEG-IFN and ribavirin. On-treatment viral dynamics and SVR achieved by the triple therapy could offer hope for improving the response in patients infected with HCV-1 in high viral loads.

Patients and Methods

From December 2005 to March 2006, 21 patients with chronic hepatitis C agreed to receive fluvastatin, in addition to standard PEG-IFN/ribavirin treatment, at the Department of Hepatology, Toranomon Hospital, Metropolitan Tokyo. They all were: (1) positive for antibody to HCV (anti-HCV) and HCV RNA of genotype 1b, and not co-infected with HCV of the other genotypes; (2) negative for hepatitis B surface antigen or antibody to human immunodeficiency virus type-1 (HIV-1); (3) confirmed within the past 2 months to have high HCV RNA levels of ≥ 100 KIU/ml, which is the Japanese definition of high viral loads [16, 17]; (4) platelet counts $>80 \times 10^3/\text{mm}^3$ and without cirrhosis diagnosed by ultrasonography; (5) body weight >40 kg and not pregnant or lactating; (6) a total alcohol intake of <500 kg during the past; (7) without HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune hepatitis; (8) without antiviral or immunosuppressive treatment during the previous 3 months, and (9) with the wish to comply to the treatment protocol for 48–72 weeks.

They were followed for liver function and virological markers at least monthly during treatment and until 24 weeks after completion of triple treatment. Informed consent was obtained from each patient, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Markers of HCV Infection

Anti-HCV was determined by third-generation enzyme-linked immunosorbent assay (ELISA) using commercial kits (Ortho HCV Ab ELISA Test 3; Chiron Cooperation, Emeryville, Calif., USA). HCV RNA was determined quantitatively by polymerase chain reaction (PCR; Cobas Amplicor HCV Monitor ver. 2.0, Roche Diagnostics, Tokyo, Japan), with a dynamic range from 5 to 5,000 KIU/ml, in sera diluted 10-fold at baseline, as well as every 2 weeks until 8 weeks after initiation of therapy and monthly thereafter. Sera negative for HCV RNA (<5 KIU/ml) by the

quantitative assay were tested by qualitative PCR (Amplicor, Roche Molecular Systems, Inc., Branchburg, N.J., USA) with the detection limit at 100 copies/ml.

Fluvastatin Added to Combined PEG-IFN and Ribavirin

Patients received subcutaneous PEG-IFN- $\alpha 2b$ (PEG-Intron, Schering-Plough Corp., Kenilworth, N.J., USA) weekly at a median dose of 1.5 (range 1.3–1.7) $\mu\text{g}/\text{kg}$, along with oral ribavirin daily at a median dose of 12.3 (range 10.2–13.7) mg/kg for 48–72 weeks. The dose of ribavirin was adjusted according to body weight: 600 mg for patients weighing ≤ 60 kg; 800 mg for those between >60 and <80 kg, and 1,000 mg for those ≥ 80 kg. In addition, the patients received 20 mg oral fluvastatin daily for 48–72 weeks.

Results

Patients Who Received Triple Treatment with Fluvastatin, PEG-IFN and Ribavirin

The baseline characteristics of the 21 patients infected with HCV-1b at a high viral load and who received triple therapy with fluvastatin, PEG-IFN and ribavirin are listed in table 1. Their median age was 56 years, and 11 (52%) were men. Their hematological and biochemical values including serum lipids were within normal limits, except for elevated levels of AST and ALT. A lack of mutations affecting the response to IFN in the core protein, i.e., mutations at positions 70 and 91 [16], and amino acid substitutions in the IFN-sensitivity determining region [18] were detected in HCV RNA from 29 and 35% of the patients.

Loss of HCV RNA from Serum in Patients with Triple Treatment

Figure 1 illustrates the cumulative loss of HCV RNA from the serum in 21 patients infected with HCV-1b at high loads. HCV RNA was cleared in 52% of the patients during the first 12 weeks. It decreased slowly thereafter, and was lost in 93% of the patients at 48 weeks. Triple treatment was continued in 6 of the 21 patients for an additional 24 weeks. HCV RNA was cleared from the serum in 5 (83%) of them at completion of the 72-week treatment.

Sustained Virological Response in Patients Receiving Triple Treatment

Of the 21 patients given triple treatment, 19 received it for 48 weeks. Skin rashes and general malaise developed in 2 of them at weeks 17 and 24, respectively, and fluvastatin was withdrawn while PEG-IFN and ribavirin were continued. Six patients continued to receive triple treatment for an additional 24 weeks. Therefore, the loss of HCV RNA from the serum 24 weeks after completion of

Table 1. Baseline characteristics of patients (n = 21) with chronic hepatitis C who received triple treatment with fluvastatin, PEG-IFN and ribavirin

	Normal ranges	Patients
Age, years	NA	56 (32–63)
Men	NA	11 (52%)
Albumin, g/dl	3.9–5.2	3.8 (3.4–4.2)
Hemoglobin, g/dl	11.3–17.0	14.4 (12.1–16.4)
Platelets, $\times 10^3/\text{mm}^3$	141–350	190 (119–240)
AST, IU/l	13–33	50 (21–208)
ALT, IU/l	6–42	64 (23–391)
γ -GTP	9–109	36 (11–301)
ICG ₁₅ , %	<10	12 (2–27)
HCV RNA, KIU/ml	NA	2,000 (14– >5,000)
Past treatments	NA	8 (36%)
Ribavirin dose, mg/kg	NA	12.3 (10.2–13.7)
Serum lipids		
Total cholesterol, mg/dl	122–240	163 (124–273)
LDL-C, mg/dl	86–160	99 (59–187)
HDL-C, mg/dl	35–75	46 (28–73)
Mutations in HCV RNA		
Wild-type at aa70	NA	12 (57%)
Wild-type at aa91	NA	10 (48%)
Double wild, aa71/aa90	NA	6 (29%)
Mutations in ISDR	NA	6/17 (35%)

Data are expressed as the number of patients with percentages in parentheses or median values with ranges in parentheses. AST = Aspartate aminotransferase; ALT = alanine aminotransferase; γ -GTP = γ -glutamyltranspeptidase; ICG₁₅ = retention of indocyanine green at 15 min; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; NA = not applicable.

therapy was assessed in the remaining 15 patients. Figure 2 shows the SVR in 15 patients. SVR was accomplished in 10 (67%) of them, including 7 of 9 (78%) male patients and 3 of 6 (50%) female patients. Of the 5 female patients aged >50 years, 2 (40%) gained SVR.

Of the 6 patients who received triple treatment for 72 weeks, SVR was accomplished in 4 (67%), including 1 of 2 male and 3 of 4 female patients who were aged 56, 58 and 62 years, respectively.

Discussion

Statins comprise a group of drugs capable of inhibiting HMG-CoA reductase and can regulate the synthesis of cholesterol by competing with the authentic substrate [19,

Fig. 1. Kaplan-Meier table illustrating the cumulative loss of HCV RNA from the serum in patients with chronic hepatitis C. The patients received fluvastatin in addition to PEG-IFN/ribavirin and were followed during 48 weeks of treatment.

Fig. 2. Sustained virological response in patients with chronic hepatitis C to the combined treatment with fluvastatin, PEG-IFN and ribavirin for 48 weeks. SVR was compared among the total, male and female patients, as well as the female patients aged more than 50 years.

20]. They are the best selling drugs in the world, and have been prescribed to more than 30 million patients for lowering serum levels of low-density lipoprotein cholesterol (LDL-C) with the main purpose of preventing cardiovascular accidents [21, 22]. The great virtue of statins is their excellent safety record. Recipients are rarely forced to discontinue statins due to mild side effects including myalgia, headache and skin rashes, thereby allowing their long-term use to be extended to decades and even for life. Liver toxicity is found in less than 1.2% of 49,275 patients with a history of cardiovascular disease [23].

In transfection experiments of cultured hepatocytes, statins inhibited the replication of HCV in synergism with IFN- α [14]. Of particular note, the extent of inhibition differs among various statins, and is highest for lipophilic fluvastatin and lowest for hydrophilic pravastatin. In clinical studies, monotherapy with fluvastatin suppressed serum HCV RNA by log 1.75 at a daily therapeutic dose of <80 mg [13], while no such effects were gained with 20 mg atorvastatin daily [24]. Hence, the ability to inhibit HCV replication may differ among diverse statins through mechanisms not directly associated with lowered levels of LDL-C; fluvastatin decreases total cholesterol and LDL-C levels to a lesser extent than the other statins in current use.

In this open-label pilot study, the response to triple treatment with fluvastatin 20 mg daily supplementing the standard PEG-IFN/ribavirin was examined in 21 patients infected with HCV-1b in high loads. The cumulative loss of HCV RNA from the serum was gained in 93% of the patients after they had received triple treatment for 48 weeks. Only 2 of the 21 (10%) patients did not tolerate fluvastatin due to skin rashes and general malaise. The triple treatment was continued for 72 weeks in 6 patients to determine its long-term effects. SVR was achieved in 10 of 15 (67%) patients on the 48-week schedule, including 7 of 9 (78%) male patients and 3 of 6 (50%) female patients. Of the 5 female patients aged >50 years, 2 (40%) gained SVR. Of the 6 patients who received triple treatment for 72 weeks, 4 (67%) achieved SVR, including 1 of 2 male and 3 of 4 female patients who were aged 56, 58 and 62 years, respectively. SVR gained by triple treatment with fluvastatin, PEG-IFN and ribavirin for 48 weeks (67%) is better than that 80 of 160 (50%) and 55 of 118 (47%) patients who received PEG-IFN and ribavirin for 48 weeks [16, 25]; they all were infected with HCV-1b at high loads.

Sex and age are the two principal host factors influencing the response to IFN-based treatment. Although women respond better to combined IFN and ribavirin than

men in large-scale multinational studies in Western countries [8, 26], target patients were rather young with the median age of about 40–41 years. Sex-dependent responsiveness to IFN is reversed with age, however, and the response to PEG-IFN/ribavirin is much poorer in female than male patients aged 50 years or older (22/100 [22%] vs. 50/94 [53%], $p < 0.001$) [10]. In view of the estrogens that can prohibit fibrosis in experimental cirrhosis induced in rats [27], levels of female sex hormones, which are lower in women than men older than 50 years [28], could be responsible for the sex difference in response to IFN of patients with chronic hepatitis C. Supplementation of aged female patients with estrogens may increase the response, but it can accelerate physiological osteoporosis [29, 30]. Since statin therapy does not increase the risk of severe hepatotoxicity in patients with chronic hepatitis C [31], fluvastatin may provide aged women with an advantage in gaining SVR to PEG-IFN/ribavirin. Furthermore, fluvastatin does not augment the side effects of other drugs, such as cyclosporine A and warfarin, because it is metabolized by CYP2C9 in the liver, unlike other statins that are disposed by CYP3A4 as other drugs [32].

Should statins interfere with the replication of HCV, it remains an open question how they do it. At least three mechanisms may be conceived for the decrease in HCV replication in patients receiving statins. HCV circulates in association with LDL-C [33], and on that basis, the LDL-C receptor has been proposed for infection of hepatocytes [34–36]. HCV RNA replicates in association with lipid droplets in hepatocytes [37, 38], and therefore, the synthesis of cholesterol blocked by statins can reduce the basis on which HCV thrives. However, these two scenarios seem to stand at odds with the response to IFN being better in patients with higher LDL-C levels [39, 40]. Statins prohibit the synthesis of mevalonate that is modified into geranylgeranyl (containing 20 carbons) and then farnesyl (15 carbons) [19]. They both prenylate most cellular proteins to make them lipophilic toward associations with membranes expressing their biological activities [41]. As an extension to these, the geranylgeranylation of host proteins, which may be required for the replication of HCV, is implicated in the capacity of statins to downregulate HCV infection [42–44]. For that matter, most pleiotropic effects of statins, including a decreased incidence of dementia and Alzheimer disease [45–47], could be associated with the inhibition of prenylation rather than lowered levels of LDL-C in the serum [48].

This study is not without limitations. The number of patients receiving fluvastatin along with PEG-IFN and ribavirin is small and fails to gain statistical power in any comparison with those given PEG-IFN/ribavirin in previous studies. The ability of other statins in improving the response to PEG-IFN/ribavirin has not been examined to discover the mechanism of how fluvastatin improves the response to antiviral treatments. The promising results obtained in this study hopefully will invite further interest for planning clinical trials with extended numbers of patients and targeting elderly women in par-

ticular. Meanwhile, it may be worth looking back in the database to examine the response of patients receiving PEG-IFN/ribavirin for chronic hepatitis C, simultaneously with statins for controlling LDL-C levels, to see if they fared better than those not taking statins routinely.

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