fluvastatin was administered orally at 20–40 mg per day [9, 10]. Statins also inhibit the synthesis of geranylgeranyl pyrophosphate from farnesyl pyrophosphate in the cholesterol synthesis pathway [11] Geranylgeranyl pyrophosphate binds to host protein and forms geranylgeranylated protein. Geranylgeranylated protein has been shown to play an important role in the replication of HCV RNA [12]. Thus, statins are considered to inhibit replication of HCV RNA by inhibiting the synthesis of geranylgeranyl pyrophosphate and the subsequent formation of geranylgeranylated protein [13]. Moreover, statins have been shown to inhibit HCV RNA replication in vitro [14, 15].

Telaprevir is a strong inhibitor of cytochrome P450 3A (CYP3A). Consequently, when telaprevir is combined with drugs metabolized by CYP3A4, the plasma concentrations of those drugs increase, which may result in an increase in adverse events. Atorvastatin, lovastatin, and simvastatin are mainly metabolized by CYP3A4, and so their combination with telaprevir is contraindicated [16]. It has also been suggested that statin plasma concentrations of fluvastatin, pitavastatin, and pravastatin increase when combined with telaprevir. Careful monitoring for adverse events during treatment is recommended [16].

The add-on effect on telaprevir-based triple combination therapy remains unknown. Based on our previous finding that the SVR rate was improved by combining fluvastatin with peg-IFN/RBV therapy, we designed the present prospective, randomized, controlled study to investigate the effect of fluvastatin on telaprevir-based combination therapy in patients with HCV genotype 1b. We also evaluated the safety of the fluvastatin add-on in peg-IFN/RBV/telaprevir therapy.

Patients and methods

Study design

This was an open-label, prospective, randomized, multicenter trial. Among 124 consecutive patients with genotype 1b, chronic hepatitis C who visited Nippon Medical School Chiba Hokusoh Hospital, Shinmatsudo General Central Hospital, Nippon Medical School and Hakujikai Memorial Hospital between December 2011 and November 2012, 116 patients met the inclusion criteria. The remaining 8 patients were excluded: 6 had thrombocytopenia and 2 did not provide written informed consent. Patients were eligible for enrollment if they fulfilled the following criteria: HCV RNA detectable in serum by real-time polymerase chain reaction (PCR); white blood cell count of more than 2,000/mm^3; platelet count of more than 500,000/mm^3; and

hemoglobin levels of more than 10 g/dl on laboratory testing before treatment initiation. The exclusion criteria were as follows: positive result for hepatitis B surface antigen and antibody to human immunodeficiency virus type-1 (HIV-1), complications by other chronic liver diseases such as autoimmune hepatitis, primary biliary cirrhosis or alcoholic hepatitis; decompensated liver cirrhosis; current development of hepatocellular carcinoma; severe renal disease; abnormal thyroid function; poorly controlled diabetes; poorly controlled hypertension; medication with Chinese herbal medicine; past medical history of interstitial pneumonia; pregnancy or possibility of pregnancy; lactating; severe depression; past medical history of allergy to biological preparations such as vaccine; medication with fibrates or statins; or past medical history of allergy to interferon, ribavirin, telaprevir or fluvastatin.

Using a random number table generated by a computer, the study patients were randomly allocated to either the group receiving peg-IFN/RBV/telaprevir without fluvastatin, provisionally designated as the control group, or the group receiving peg-IFN/RBV/telaprevir with fluvastatin, provisionally designated as the fluvastatin group.

The study protocol was prepared following ethical guidelines established in conformity with the 2004 Declaration of Helsinki after approval by the Ethics Committees of Nippon Medical School Chiba Hokusoh Hospital (No. 523029) and Shinmatsudo Central General Hospital. All patients provided written informed consent.

Treatment and definition of virological response

All patients received combination therapy with peg-IFNα2b (PEGINTRON; MSD, Tokyo, Japan), ribavirin (REBETOL; MSD, Tokyo, Japan) and telaprevir (TELAVIC®, Mitsubishi Tanabe Pharma, Osaka, Japan) for 12 weeks, followed by 12 weeks of peg-IFNα2b and ribavirin. Peg-IFNα-2b was injected subcutaneously 1.5μg/kg once weekly. The patients received oral administration of ribavirin. Ribavirin dose was adjusted by body weight (600 mg, 800 mg, and 1000 mg per day for <60 kg, 60–80 kg, and >80 kg, respectively) based on the guidelines of the Ministry of Health, Labor and Welfare of Japan. Telaprevir at a dose of 750 mg was administered every 8 hours after meals. The doses were appropriately reduced when an adverse event such as anemia, skin rash, or renal insufficiency occurred during the treatment course. Fluvastatin was orally administered at 30 mg/day for 24 weeks. When HCV RNA was undetectable at 4 and 12 weeks after the initiation of treatment, patients were considered to have achieved

rapid virological response (RVR) and complete early virological response (cEVR), respectively. Patients who tested undetectable for HCV RNA at the time of treatment completion were judged as having achieved end of treatment response (ETR). The patients were followed for 24 weeks after treatment completion. Patients who were found undetectable for HCV RNA at 24 weeks after treatment completion were judged as SVR. Patients who exhibited an ETR but in whom the virus was detected at 24 weeks after completion of treatment were considered to have a relapse. Patients who failed to achieve HCV RNA negativity by the end of treatment were considered as having non-virological response (NVR).

Laboratory tests

Peripheral blood examination, liver function tests and renal function tests were performed weekly until 24 weeks after treatment initiation and then monthly until 24 weeks after completion of treatment. For biochemical tests before treatment initiation, data were obtained in the fasting state. HCV RNA levels were measured using real-time PCR (COBAS® AmpliPrep; Roche Diagnostics, Tokyo, Japan). Gene mutations in the core amino acids 70 and 91, and NS5A regions (interferon sensitivity determining region; ISDR) of the HCV genome were determined using the direct sequencing method.

Core amino acid 70 was defined as wild type (arginine) or mutant type (glutamine or histidine), and core amino acid 91 was defined as wild type (leucine) or mutant type (methionine). Amino acid mutations in the ISDR were defined as wild type (0, 1) or mutant type (others). Genomic DNA was extracted from whole blood using a DNA isolation kit on the MagNA Pure LC Instrument (Roche Diagnostics, Basel, Switzerland). Single nucleotide polymorphisms (SNPs) at rs8099917, which is located in the locus adjacent to the interleukin 28B (IL28B) gene on chromosome 19, were determined using real-time PCR using TaqMan® SNP Genotyping Assays on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The rs8099917 genotype was classified into two categories: TT (major genotype) and non-TT (minor genotype: TG or GG). SNPs at rs1127354, which is located in the locus adjacent to the inosine triphosphatase (ITPA) gene, were genotyped by real-time detection PCR using the TaqMan® SNP Genotyping Assay and the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The rs1127354 genotype was classified into two categories: CC (major genotype) and non-CC (minor genotype: CA or AA).

Safety assessments

During the on-treatment period, all patients underwent chemical and hematologic

assessment and were monitored for safety through every hospital visit, from the start of dosing to 4 weeks after the last dose of the study drug was administered. **Furthermore, adverse events, including statin-induced rhabdomyolysis, were carefully monitored in consideration of possible interactions between Telaprevir and fluvastatin.**

Statistical analysis

The planned sample size was based on the assumption that the SVR rate would be 70% in the control group, and 85% in the fluvastatin group, resulting in a necessary sample size of 240 patients with a two-sided significance level of 5%, and statistical power of 80%. Fisher's exact test and the Mann-Whitney *U*-test were employed for comparison of the baseline and on-treatment factors. Fisher's exact test was employed for comparison of RVR, cEVR, ETR, and SVR rates between the groups. Statistical analysis was performed using SPSS version 17.0 (IBM Japan, Tokyo, Japan). The level of significance was set at $p < 0.05$.

Results

Patient characteristics

Of 124 patients with chronic hepatitis C who were screened as study candidates, 116

patients were eligible for this prospective, controlled study. There were 56 males and 60 females aged 28-71 years (median, 60 years). One hundred twelve patients completed the therapy as scheduled and were subjected to analysis (intention-to-treat analysis). The remaining 4 patients (one with depression and one with anemia in the fluvastatin group; one with severe skin rash (grade 3) and one with severe appetite loss in the control group) discontinued therapy, but were included in the analysis. The control and fluvastatin groups each consisted of 58 patients. No significant differences between the two groups were noted with respect to patient background data such as gender, age, previous treatment response, peripheral blood counts, core amino acid 70 and 91 substitutions, IL28B genotype and ITPA genotype (Table 1).

Virological response

Overall RVR, cEVR, ETR, and SVR rates were 81.9% (95/116), 97.4% (112/115), 97.4% (113/116), and 82.8% (96/116) in all 116 patients, respectively. The RVR, cEVR, ETR, and SVR rates in the control group were 87.9% (51/58), 96.5% (55/57), 96.6% (56/58), and 84.5% (49/58), respectively, while the rates in the fluvastatin group were 75.9% (44/58), 98.3% (57/58), 98.3% (57/58), and 81.0% (47/58), respectively. The differences in the RVR, cEVR, ETR and SVR rates were not statistically significant

between the control group and the fluvastatin group (Fig. 1). We next compared the SVR rate between the control and fluvastatin groups, stratifying baseline factors such as gender and IL28B genotype. No factors associated with the SVR rate were found to show a statistically significant difference between the two groups (Fig. 2).

Drug adherence and drug interactions

The effects of drug dosage in the two groups were also analyzed. The median dose of telaprevir was 130500 mg (range, 42000-189000) in all patients, 126000 mg (range, 66000-189000) in the control group, and 143250 mg (range, 42000-189000) in the fluvastatin group. There was no statistically significant difference in the dose of telaprevir between the groups (p=0.152). In investigating the dosage of PEG-IFNα2b, the median dose was found to be 1.51 μg/kg/week (range, 0.83-1.92) in all patients, 1.52 μg/kg/week (range, 0.83-1.92) in the control group, and 1.50 μg/kg/week (range, 1.07-1.88) in the fluvastatin group. There was no statistically significant difference between the groups (p=0.211). Investigation of the dosage of ribavirin revealed that the median dose was 7.89 mg/kg/day (range, 3.55-14.80) in all patients, 8.62 mg/kg/day (range, 4.01-12.21) in the control group, and 7.43 mg/kg/day (range, 3.55-14.80) in the fluvastatin group. The dose was significantly lower in the fluvastatin group (p=0.048).

Next, the drug interactions between telaprevir and fluvastatin were investigated. Since the plasma concentrations of fluvastatin and telaprevir could not be measured, the changes in LDL-cholesterol in the fluvastatin group were investigated. In the present study, LDL-cholesterol in the fluvastatin combination group was 97 mg/dl (range, 21-189) at the start of treatment and 76mg/dl (range, 25-129) during treatment. The decrease rate was 24.7%, comparable to the normal administration of fluvastatin alone (20-30%) [9, 10].

Adverse events

A summary of adverse events is shown in Table 2. Adverse events occurred in many of the study patients. Adverse events such as severe anemia, mild anemia, skin rash and eruption, renal disorders, increase in serum uric acid, gastrointestinal disorders including nausea and appetite loss, and psychiatric disorders including insomnia and depression were similar between the two groups. Anemia was the most common clinical adverse event in both groups. Anemia was classified as severe anemia (hemoglobin levels of less than 8.5g/dl) and mild anemia (hemoglobin levels of less than 10.0g/dl). All of the patients with mild and severe anemia required dose reductions in RBV or

telaprevir. Only one patient in the fluvastatin group discontinued the study due to severe anemia, and after the completion of treatment the patient had a relapse. Increase in serum uric acid was well controlled with administration of allopurinol or febuxostat. One patient in the control group stopped the study at week 7 due to severe skin rash (grade 3); nevertheless, the patient achieved SVR. No adverse events, such as rhabdomyolysis, were associated with fluvastatin. There were no deaths in the study.

Discussion

The present study is the first prospective, randomized trial to be conducted on the use of a statin combined with peg-IFN/RBV/telaprevir therapy for genotype 1b chronic hepatitis C. Previously, we reported that combining fluvastatin with peg-IFN/RBV therapy for chronic hepatitis C with genotype 1b, high viral load reduced the post-treatment relapse rate, and, as a result, increased the SVR rate [7, 8]. Kohjima et al. and Bader et al. also reported the effects of an add-on statin to peg-IFN/RBV in chronic hepatitis C [17, 18]. The effect of peg-IFN/RBV therapy combined with a statin was subsequently investigated in a meta-analysis, and favorable results were shown [19]. However, up until now, there have been no reports on how a statin affects peg-IFN/RBV therapy in combination with a protease inhibitor such as telaprevir, which is the

mainstream of treatment for chronic hepatitis C with genotype 1b.

The present study found no add-on effect from the combination of fluvastatin with peg-IFN/RBV/telaprevir therapy for HCV genotype 1b-infected chronic hepatitis C. Several reasons may be considered for this result. In our previously reported trial of fluvastatin combined with peg-IFN/RBV therapy, the patients in whom an add-on effect of fluvastatin was seen included male patients, patients with pretreatment relapse, patients with IL28B major genotype, and patients with core amino acid 70 wild type. In other words, an add-on effect of fluvastatin was thought to occur in patients who tended to respond to peg-IFN/RBV therapy [7]. However, it has been reported that there is a high probability (over 80%) that IL28B major genotype patients in particular, who receive peg-IFN/RBV therapy combined with telaprevir, will achieve SVR [4-6]. Improving on this very high response rate is considered difficult. The next reason for finding no add-on effect from the combination of fluvastatin with peg-IFN/RBV/telaprevir therapy relates to reports that ETR can be achieved in many IL28B minor genotype patients [1, 4, 5, 20]. Similar results were obtained in the present study. In a previous report, the results of a trial of fluvastatin combined with peg-IFN/RBV therapy showed suppression of the relapse rate after treatment

completion [8]. Thus, in the present study, we expected that the relapse rate might have been suppressed after ETR in the IL28B minor genotype patients; unfortunately, no such suppression was seen. As a result, in patients with the IL28B major genotype, for whom a statin add-on effect is expected, the treatment outcome is not improved since a sufficient effect is already obtained with peg-IFN/RBV/telaprevir without fluvastatin. In the IL28B minor genotype patients, in whom the effect of peg-IFN/RBV therapy combined with telaprevir is insufficient, it may be concluded that essentially no statin add-on effect can be expected.

It has also been previously reported that the SVR rate improved with the combination of pitavastatin and eicosapentaenoic acid (EPA) added to peg-IFN/RBV therapy for genotype 1b chronic hepatitis C, high viral load [17]. In particular, a significant improvement in the SVR rate was reported in patients with the IL28B minor genotype, which is resistant to treatment. Points that differed from our study [7] were that the statin formulation was different and EPA was used. A reported advantage of using EPA is that the expression of LDL receptors, which is strengthened with pitavastatin, is suppressed with the use of EPA [17]. This phenomenon is very important since one route of HCV infection of cells occurs via LDL receptors. The above indicates that the

SVR rate in difficult-to-treat patients, such as patients with the IL28B minor genotype, may be improved with combination therapy consisting of a statin and EPA together with peg-IFN/RBV therapy plus telaprevir. It will be interesting to see whether these add-on therapies to the combination of the next generation protease inhibitor and peg-IFN/RBV [21, 22], which may be available for clinical use in the future, will contribute to improving the SVR rate.

With the use of statins, there is concern of drug-drug interactions with protease inhibitors. Simvastatin, lovastatin, and atorvastatin, like telaprevir, are metabolized mainly via CYP3A4. Consequently, these statins are contraindicated for use with telaprevir [16]. Meanwhile, although the fluvastatin administered in the present study is metabolized by several enzymes, including CYP2C9, CYP3A4, and CYP2C8, it is metabolized mainly by CYP2C9 [23]. It is also reported that fluvastatin is metabolized by CYP3A4 at a high concentration (200 μM) and by CYP2C9 at a low concentration (up to 0.2 μM) [24]. In fact, the maximum blood concentration when fluvastatin is administered orally at 20–40 mg per day is about 0.35 μM, close to the low concentration (up to 0.2 μM). In the present study, the telaprevir dosage and frequency of adverse events were comparable between the

fluvastatin and non-fluvastatin groups. However, since the blood concentration of telaprevir was not measured, it is not known whether there was actually no drug-drug interaction. The LDL-cholesterol decreasing effect of fluvastatin in this study was 24.7%, equivalent to that when fluvastatin is administered alone. Considering the above, there does not seem to be an increase in adverse events with the combined use of fluvastatin.

This study had several limitations. First, the sample size was small and may have been inadequate for analysis. The target number of cases was 240, but an interim analysis indicated a high possibility that no statin add-on effect was obtained, and entry was discontinued. In fact, mathematical analysis also showed that the SVR rate was not higher in the fluvastatin group than in the non-fluvastatin group. The second limitation is that the dosage of fluvastatin was not investigated. Future investigation will be needed to establish appropriate dosages.

Previously reported factors contributing to SVR in triple therapy with telaprevir are previous treatment response, HCV core amino acid 70 substitution, IL28B genotype, and presence of RVR [4, 5 6, 25]. These factors include items equivalent to those in the

present study. The only independent baseline factor contributing to SVR in our study was found to be the IL28B genotype. The SVR rate was very good at 97.2% (69/71) in patients with the IL28B major genotype, but was inadequate at 60.0% (27/45) in patients with the IL28B minor genotype. Although the presence or absence of RVR is an on-treatment factor, it was identified as an independent factor. It was shown that, in patients for whom IL28B status was unknown prior to treatment, the presence or absence of RVR was a very important factor in the acquisition of SVR.

In conclusion, administration of fluvastatin with telaprevir/peg-IFN/RBV was a safe combination in this trial. However, this prospective, open-label, randomized, controlled trial showed that fluvastatin had no add-on effect on telaprevir-based triple combination therapy for chronic hepatitis C patients with HCV genotype 1b.

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Conflict of interest

The authors have no conflicts of interest to declare.

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