long enough to achieve a high SVR. For these reasons, especially in genotype 2 infection, it is necessary to identify those patients who could achieve SVR with a shorter treatment course (≤16 weeks) to free them of unnecessary side effects and reduce costs, preferably as early as possible [6–8]. Furthermore, we also sometimes encounter treatment-resistant patients infected with genotype 2a [3, 10]. The underlying mechanism(s) of the different virological responses to treatment in patients infected with genotype 2a is still unclear. Hence, the pretreatment predictors of the efficacy of IFN + ribavirin combination therapy were investigated in the present study.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of infected patients with genotype 1b and a high viral load (≥100 kIU/ml) are predictors of poor virological response to 48- and 72-week pegylated IFN (PEG-IFN) + ribavirin combination therapies [11–15], and also affect clinical outcomes, including insulin resistance and hepatocarcinogenesis [16–18]. However, it is unknown whether the aa substitutions of the core region in patients infected with genotype 2a who have high viral load might also be as useful as the pretreatment virological predictive factors apart from the genotype and viral load.

The present study included 154 Japanese adults with genotype 2a and a high viral load, who received combination therapy for 24 weeks. The aim of the study was to investigate the treatment efficacy and pretreatment predictive factors including virological features.

Materials and Methods

Study Population

A total of 190 HCV genotype 2a-infected Japanese adult patients were consecutively recruited into the study protocol of the combination therapy with IFN (PEG-IFNα-2b or IFNα-2b) + ribavirin for 24 weeks between March 2002 and February 2008 at Toranomon Hospital, Tokyo, Japan. Among these, 154 patients, who could complete a total of 24 weeks of combination therapy, were enrolled in this retrospective study and fulfilled the following criteria: (1) They were negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emerville, Calif., USA), and positive for HCV-RNA qualitative analysis with PCR (Amplicor, Roche Diagnostic Systems, Calif., USA). (2) They were naive to ribavirin therapy. (3) They were infected with HCV genotype 2a alone. (4) Each had a high viral load (≥100 kIU/ml) by quantitative analysis of HCV-RNA with PCR (Amplicor GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems, Inc., Pleasanton, Calif., USA) within the preceding 2 months of enrolment. (5) They had no hepatocellular carcinoma. (6) Their body weight was >40 kg. (7) All were free of coinfection with human immunodeficiency virus. (8) None had been treated with antiviral or immunosuppressive agents within the preceding 3 months of enrolment. (9) None was an alcoholic; lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (10) None had other forms of hepatitis, such as hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (11) None of the females were pregnant or lactating mothers. (12) All patients had completed a 24-week follow-up program after cessation of treatment, and SVR could be evaluated. (13) Each signed a consent form of the study protocol that had been approved by the Human Ethics Review Committee.

Table 1 summarizes the profiles and data of the 154 patients at the commencement of combination therapy with IFN + ribavirin. The study included 92 men and 62 women, aged 20–70 years (median 53). In all patients, the total duration of treatment was 24 weeks. In 43 of the 154 (27.9%) patients, the dose of ribavirin was reduced during treatment due to a fall in Hb concentration.

With regard to the treatment protocol, 104 (67.5%) patients received PEG-IFNα-2b + ribavirin for 24 weeks, and the remaining 50 (32.5%) patients received IFNα-2b + ribavirin for 24 weeks. They received PEG-IFNα-2b at a median dose of 1.5 μg/kg (range 1.0–1.8) subcutaneously each week, or IFNα-2b at a median dose of 6 million units (range 3–6) intramuscularly each day (7 times per week for the initial 2 weeks, followed by 3 times per week for 22 weeks). They also received oral ribavirin at a median dose of 11.2 mg/kg (range 5.4–14.1) daily.

The treatment efficacy was evaluated by HCV-RNA positivity based on qualitative PCR analysis at the end of treatment (nonvirological response; NVR), and by HCV-RNA negativity based on qualitative PCR analysis at 24 weeks after the completion of therapy (SVR). Furthermore, rapid responders were defined as SVR patients who could achieve a negative status within 8 weeks after the start of treatment, based on qualitative PCR analysis.

Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for alanine aminotransferase (ALT) and HCV-RNA levels. The serum samples were frozen at –80° within 4 h of collection and thawed at the time of measurement. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of the NS5 region [19]. HCV-RNA levels were measured by quantitative PCR (Amplicor GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems, Inc.) at least once every month before, during, and after therapy. The dynamic range of the assay was 5–5,000 kIU/ml. Samples collected during and after therapy that showed undetectable levels of HCV-RNA (<5 kIU/ml) were checked by qualitative PCR (Amplicor HCV v2.0, Roche Molecular Systems, Inc.), which has a higher sensitivity than quantitative analysis, and the results were expressed as positive or negative. The lower limit of the assay was 50 IU/ml.

Histopathological Examination of Liver Biopsies

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens

Table 1. Profile and laboratory data of 154 patients infected with HCV genotype 2a

Demographic data	
Number of patients	154
Sex, M/F	92/62
Age, years*	53 (20–70)
History of blood transfusion	43 (27.9%)
Family history of liver disease	26 (16.9%)
Body mass index*	22.7 (17.9–31.8)
Laboratory data*	
Serum aspartate aminotransferase, IU/l	43 (7–404)
Serum alanine aminotransferase, IU/l	54 (8–651)
Serum albumin, g/dl	3.9 (3.2–4.7)
γ-Glutamyl transpeptidase, IU/l	38 (9–406)
Leukocytes, /mm^3	4,800 (2,200–9,000)
Hemoglobin, g/dl	14.4 (9.9–17.8)
Platelet count, × 104/mm^3	17.9 (6.1–32.9)
Indocyanine green retention rate at 15 min, %	13 (4–35)
Serum iron, μg/dl	136 (22–336)
Serum ferritin, μg/l	124 (<10–820)
Level of viremia, kIU/ml	720 (5–>5,000)
α-Fetoprotein, μg/l	4 (2–103)
Total cholesterol, mg/dl	174 (107–275)
High-density lipoprotein cholesterol, mg/dl	47 (15–109)
Low-density lipoprotein cholesterol, mg/dl	105 (48–201)
Triglycerides, mg/dl	98 (36–449)
Uric acid, mg/dl	5.6 (2.5–9.4)
Fasting plasma glucose, mg/dl	93 (75–187)
Histological findings	
Stage of fibrosis (F1/F2/F3/ND)	58/23/16/57
Grade of activity (A1/A2/ND)	57/40/57
Hepatocyte steatosis (<5% (absent)/≥5% (present)/ND)	35/52/67
Treatment	
PEG-IFNα-2b/IFNα-2b	104/50
Ribavirin dose, mg/kg*	11.2 (5.4–14.1)
Total duration of treatment, weeks	24
Past history of IFN monotherapy	56 (36.4%)

Data are number and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values. ND = Not determined.

for examinations contained ≥6 portal areas. Histopathological diagnosis was confirmed by an experienced liver pathologist (H. K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on histological assessment according to the scoring system of Desmet et al. [20].

Nucleotide Sequencing of the Core Region

We determined the sequences of aa 1–191 in the core region by the direct sequencing method using pretreatment sera of patients who could be analyzed due to adequate serum samples obtained at the start of combination treatment. These sequences were compared with the consensus sequences of genotype 2a, which were determined by comparing the sequences obtained in this study and prototype sequence (HCV J6) [21]. HCV-RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids were amplified by nested PCR using the following primers. Nucleotide sequences of the core region: the first-round PCR was performed with 2ACF5 (sense, 5′-GCA AGA CTG CTA GCC GAG TA-3′) and 2ACR6 (antisense, 5′-ATC TGA GCT GCG AGC ATC AC-3′) primers, and the second-round PCR with 2ACF3N (sense, 5′-CCT TGT GGT ACT GCC TGA TA-3′) and 2ACR8 (antisense, 5′-CCA GGT GAT GCT GTC ATT AG-3′) primers. All samples were initially denatured at 95° for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 s at 95°, annealing of primers for 30 s at 55°, and extension for 1 min at 72° with an additional 7 min for extension. Then 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR prim-

Table 2. Factors associated with sustained virological response to combination therapy with IFN + ribavirin for 24 weeks in 154 patients infected with HCV genotype 2a, identified by multivariate analysis

Factor	Category	Odds ratio (95% CI)	p
Age, years	1: ≥50	1	
	2: <50	6.37 (1.76–23.3)	0.005
Serum albumin, g/dl	1: <3.9	1	
	2: ≥3.9	3.19 (1.17–8.73)	0.024
Level of viremia, kIU/ml	1: ≥1,000	1	
	2: <1,000	2.86 (1.11–7.41)	0.030

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 3. Amino acid substitutions in the core region in non-SVR and rapid response to combination therapy with IFN + ribavirin for 24 weeks in 86 patients infected with HCV genotype 2a

	Non-SVR (n = 25)	Rapid response (n = 61)	p*
Presence of substitution site			
aa 4	1 (4.0%)	15 (24.6%)	0.032
aa 23	2 (8.0%)	0 (0%)	0.082
aa 70	1 (4.0%)	0 (0%)	NS
aa 91	0 (0%)	4 (6.6%)	NS
aa 110	11 (44.0%)	34 (55.7%)	NS

* Non-SVR vs. rapid response (Fisher's exact probability test; statistical significance ($p < 0.05$), marginal significance ($p < 0.10$)). aa = Amino acid; SVR = sustained virological response; NS = not significant.

ers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (PerkinElmer, Tokyo, Japan).

To avoid false-positive results, the procedures recommended by Kwok and Higuchi [22] to prevent contamination were strictly applied to these PCR assays. No false-positive results were observed in this study.

Statistical Analysis

Non-parametric tests were used to analyze the aa substitutions in HCV core between the groups, including the Mann-Whitney U test, χ^2 test and Fisher's exact probability test. Uni- and multivariate logistic regression analyses were used to determine the factors that significantly contributed to SVR and rapid response. We also calculated the odds ratios and 95% confidence intervals (CI). All p values <0.05 calculated by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. Potential predictive factors associated with SVR included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), ALT, albumin, γ-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelets, indocyanine green retention rate at 15 min (ICG R15), iron, ferritin, level of viremia, α-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, fasting blood glucose, type of IFN, ribavirin dose/body weight, and past history of IFN monotherapy. Furthermore, in addition to potential predictive factors associated with SVR, potential predictive factors associated with a rapid response also included aa substitution in the core region. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, Ill., USA).

Results

Virological Response Rates to Combination Therapy

The virological response could be evaluated in all 154 patients. SVR was achieved in 127 of 154 (82.5%) patients, and rapid response in 113 of 127 (90.0%). Only 5 of 154 (3.2%) patients were considered NVR.

Predictive Factors Associated with SVR in Multivariate Analysis

We then analyzed the data of all 154 patients to determine those factors that could predict SVR. Univariate analysis identified 5 parameters associated with SVR that achieved statistical significance or marginal significance. These included age (<50 years; $p < 0.001$), serum albumin (≥3.9 g/dl; $p = 0.003$), level of viremia (<1,000 kIU/ml; $p = 0.049$), history of blood transfusion (absent; $p = 0.064$), and ALT (≥30 IU/l; $p = 0.088$).

Multivariate analysis identified 3 parameters that independently influenced SVR, including age (<50 years; $p = 0.005$), serum albumin (≥3.9 g/dl; $p = 0.024$), and level of viremia (<1,000 kIU/ml; $p = 0.030$) (table 2).

Fig. 1. Sequences of aa 1–30 and aa 61–110 in the core region at the commencement of combination therapy in 86 patients infected with high HCV viral load genotype 2a. Dashes indicate aa identical to the consensus sequence of genotype 2a, and substituted aa are shown by standard single-letter codes. The aa patterns at positions that are probably associated with sensitivity to therapy are shown in boldface characters. NSR = Non-SVR; RR = rapid response.

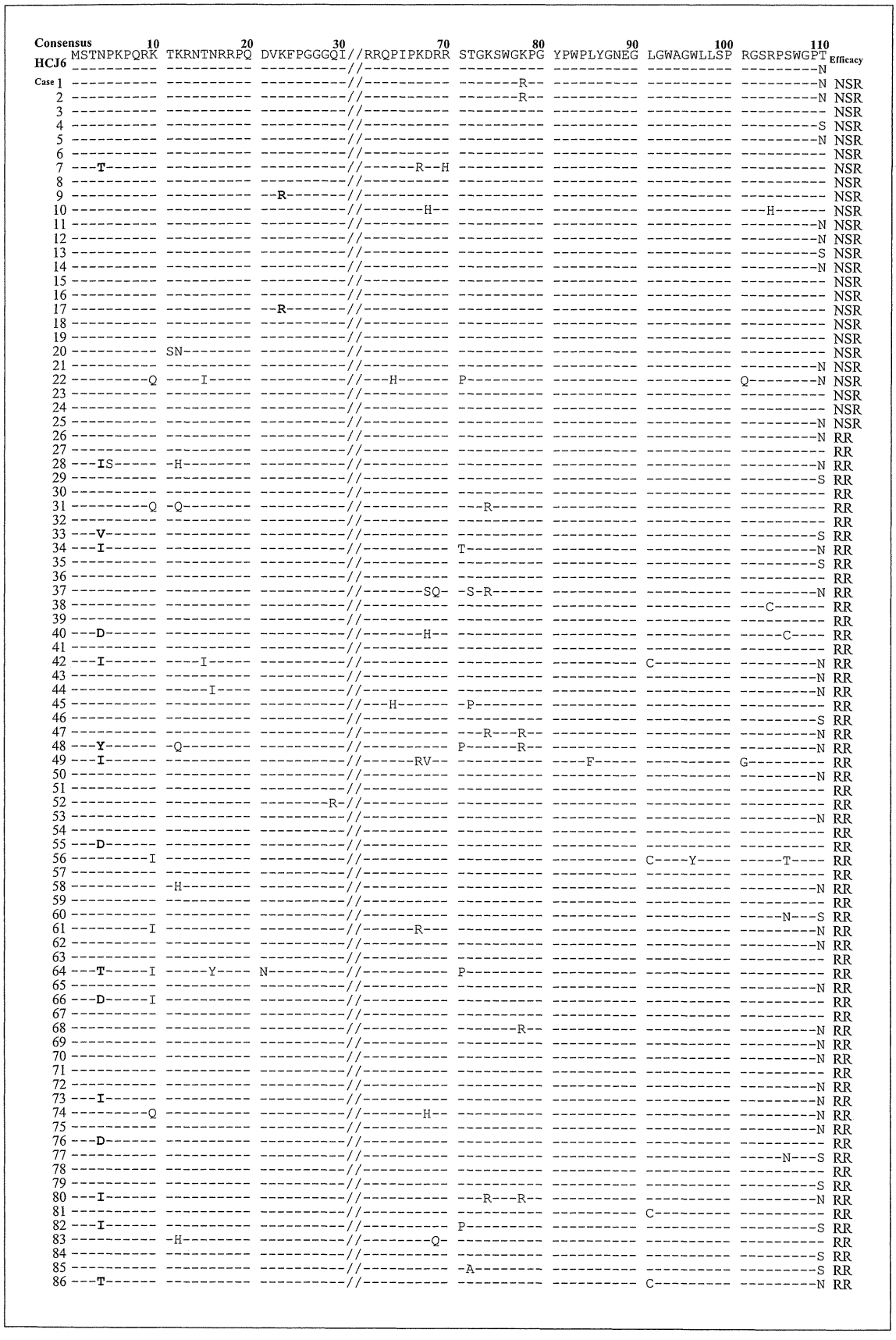
Consensus 10 20 30 70 80 90 100 110
HCJ6 MSTNPKPQRK TKRNTNRRPQ DVKFPGGGQI//RRQPIPKDRR STGKSWGKPG YPWPLYGNEG LGWAGWLLSP RGSRPSWGPT Efficacy
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Table 4. Patient profile and laboratory data of non-SVR and rapid response to combination therapy with IFN + ribavirin for 24 weeks in 86 patients infected with HCV genotype 2a, who could be analyzed by the nucleotide sequences of the core region

	Non-SVR	Rapid response	p[a]
Demographic data			
Number of patients	25	61	
Sex, M/F	12/13	39/22	NS
Age, years*	58 (34–64)	51 (20–66)	0.006
History of blood transfusion	10 (40.0%)	15 (24.6%)	NS
Family history of liver disease	4 (16.0%)	10 (16.4%)	NS
Body mass index*	23.1 (19.5–30.0)	22.7 (17.9–31.1)	NS
Laboratory data*			
Serum aspartate aminotransferase, IU/l	31 (19–200)	42 (7–125)	NS
Serum alanine aminotransferase, IU/l	44 (14–357)	53 (8–280)	NS
Serum albumin, g/dl	3.8 (3.2–4.2)	3.9 (3.2–4.5)	0.005
γ-Glutamyl transpeptidase, IU/l	38 (14–141)	39 (10–406)	NS
Leukocytes, /mm^3	4,600 (3,100–8,000)	4,800 (2,400–9,000)	NS
Hemoglobin, g/dl	14.2 (12.5–17.8)	14.7 (11.1–17.2)	NS
Platelet count, $\times 10^4$/mm^3	16.3 (8.0–32.9)	18.0 (10.6–30.6)	NS
Indocyanine green retention rate at 15 min, %	16 (5–26)	12 (6–35)	NS
Serum iron, μg/dl	152 (30–284)	144 (26–304)	NS
Serum ferritin, μg/l	133 (16–756)	123 (10–820)	NS
Level of viremia, kIU/ml	1,200 (93–>5,000)	680 (5–4,600)	0.053
α-Fetoprotein, μg/l	5 (2–103)	5 (2–48)	NS
Total cholesterol, mg/dl	172 (117–236)	184 (137–264)	NS
High-density lipoprotein cholesterol, mg/dl	49 (27–82)	49 (15–101)	NS
Low-density lipoprotein cholesterol, mg/dl	102 (48–150)	109 (73–198)	NS
Triglycerides, mg/dl	108 (55–276)	97 (39–418)	NS
Uric acid, mg/dl	5.2 (3.2–8.7)	5.7 (2.5–8.7)	NS
Fasting blood glucose, mg/dl	92 (80–126)	93 (77–109)	NS
Treatment			
Ribavirin dose, mg/kg*	11.1 (8.0–12.9)	11.2 (7.3–14.0)	NS
Past history of IFN monotherapy	9 (36.0%)	19 (31.1%)	NS

Data are number and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values.

[a] Non-SVR vs. rapid responder (Mann-Whitney U test or χ^2 test; statistical significance ($p < 0.05$), marginal significance ($p < 0.10$)). SVR = Sustained virological response; NS = not significant.

Treatment Efficacy according to Substitution Patterns in Amino Acids of the HCV Core Region

To examine the differences in virological characteristics between non-SVR and rapid response, 86 patients (25 of 27 non-SVR patients and 61 of 113 rapid responders) could be analyzed by the nucleotide sequences of HCV core region due to adequate serum samples obtained at the start of combination treatment. Figure 1 shows the sequences of aa 1–30 and aa 61–110 of the core region in 86 patients at the commencement of combination therapy. Substitutions at aa 4 (non-asparagine) of HCV core were significantly more frequent in rapid response (n = 15, 24.6%) than non-SVR (n = 1, 4.0%) patients (p = 0.032). Inversely, substitutions at aa 23 (non-lysine) were more frequent in non-SVR (n = 2, 8.0%) than rapid response (n = 0, 0%) patients (p = 0.082). There were no significant differences in the other substitution sites, including aa 70, aa 91, and aa 110 of the previous report [11], concerning the treatment efficacy of rapid response and non-SVR (table 3).

Predictive Factors Associated with Rapid Response in Multivariate Analysis

We then evaluated the data of all 86 patients who could be analyzed by the nucleotide sequences of core region to determine those factors that could predict rapid re-

Table 5. Factors associated with rapid response to combination therapy with IFN + ribavirin for 24 weeks in 86 patients infected with HCV genotype 2a, identified by multivariate analysis

Factor	Category	Odds ratio (95% CI)	p
Age, years	1: ≥50	1	
	2: <50	7.46 (1.79–31.3)	0.006
Level of viremia kIU/ml	1: ≥1,000	1	
	2: <1,000	4.33 (1.36–13.9)	0.013
Substitution of aa 4	1: asparagine	1	
	2: non-asparagine	9.97 (1.12–89.0)	0.039

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

sponse. Univariate analysis identified 5 parameters associated with rapid response that achieved statistical significance or marginal significance. As potential predictors of rapid response, table 4 indicates age (<50 years; $p = 0.006$), serum albumin (≥3.9 g/dl; $p = 0.005$), and level of viremia (<1,000 kIU/ml; $p = 0.053$). Furthermore, table 3 shows aa substitution of the core region in the pretreatment sample (substitution of aa 4 [non-asparagine], $p = 0.032$, and aa 23 [lysine], $p = 0.082$).

Multivariate analysis identified 3 parameters that independently influenced rapid response, including age (<50 years; $p = 0.006$), level of viremia (<1,000 kIU/ml; $p = 0.013$), and substitution of aa 4 (non-asparagine; $p = 0.039$) (table 5).

Discussion

Previous reports indicated that viral factors (e.g., viral load, aa substitutions in the NS5A region, early viral kinetics, and periods from the start of treatment to initial point of undetectable HCV-RNA) and host factors (e.g., body mass index, fibrosis stage, and level of soluble interleukin-2 receptor) might be important predictors of treatment response to 24-week IFN + ribavirin combination therapy in patients infected with HCV genotype 2a, in addition to treatment-related factors (e.g., treatment duration and ribavirin dose) [6–9, 23–28]. Using multivariate analysis, the present study identified viral- (viral load and substitution of aa 4) and host-related factors (age and serum albumin levels as surrogate markers of liver fibrosis [3, 11]) that influenced the virological response to 24-week combination therapy in patients with genotype 2a infection and a high viral load. IFN + ribavirin combination therapy carries potential serious side effects and is costly, especially when used long enough to achieve a high SVR. For these reasons, especially in genotype 2 infection, it is necessary to identify those patients who could achieve SVR with a shorter treatment course (≤16 weeks) to free them of unnecessary side effects and reduce costs, preferably as early as possible [6–8]. Identification of these viral and host factors before the start of combination therapy should help design better therapeutic regimens.

Amino acid substitutions at position 70 and/or 91 in the core region of patients with genotype 1b infection and a high viral load are predictors of poor virological response to 48- and 72-week PEG-IFN + ribavirin combination therapy [11–15] and also affect clinical outcomes, including insulin resistance and hepatocarcinogenesis [16–18]. This study, based on 24-week combination therapy in patients with genotype 2a infection and a high viral load, identified substitution of aa 4 in the core region as the significant determinant of treatment efficacy, but did not identify substitutions of aa 70, aa 91, and aa 110. These discrepant findings might be due to the difference of genotype and treatment duration. Other mechanisms could be also explained by the small number of NVR patients with genotype 2a (only 3%), compared with about 25% of patients infected with genotype 1b [11]. Previous studies reported that the core region might be associated with resistance to IFN monotherapy involving the Jak-STAT signaling cascade [29–32]. The present result could also be interpreted to mean that aa substitutions in the core region are associated with those proteins involved in resistance to IFN monotherapy, such as SOCS proteins, which are known to inhibit IFN-α-induced activation of the Jak-STAT pathway and expression of the antiviral proteins 2′,5′-OAS and MxA [33]. Furthermore, this result also indicates that aa substitutions in the core region might serve as surrogate markers for other proteins associated with resistance to the antiviral actions of IFN. One limitation of this study based on the small number of patients was that only the nucleotide sequences of rapid response within SVR patients were analyzed, although all of SVR patients should have been investigated (e.g., possible type II error). Further large-scale studies that examine the structural and functional impacts of aa substitutions during combination therapy should be conducted to confirm the above findings.

In conclusion, our results suggest that the aa substitution pattern in the core region in patients with a high viral load of HCV genotype 2a may partly affect the viro-

logical response to combination therapy. The limitations of this study were that it did not investigate other viral factors, such as the number of substitutions in aa 2193–2228 (the region corresponding to the IFN sensitivity-determining region [ISDR] of genotype 1b [34–36]) or aa 2163–2228 of NS5A in genotype 2a [10, 26, 37], the geographic diversities of the genotype 2a core region (distribution of consensus sequence), and other races apart from Asians in Japan. Further prospective studies, matched for aa substitutions of the core region and large groups of patients of different races, are required to determine the virological response to 24-week IFN + ribavirin combination therapy in patients with a high viral load of HCV genotype 2a.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

The Efficacy of Interferon-beta Monotherapy for Elderly Patients with Type C Hepatitis of Genotype 2

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Abstract

Objective The aim of this study was to elucidate the efficacy of interferon (IFN)-beta monotherapy for elderly patients of ≥70 years with type C hepatitis (HCV) of genotype 2.

Methods The present study was a retrospective cohort study. Inclusion criteria were type C hepatitis patients with HCV genotype 2a or 2b, ≥70 years, and IFN-beta monotherapy of within 24weeks. Thirty-one consecutive patients who satisfied the above criteria were enrolled in the present study. Independent factors that might have influenced the sustained virological response (SVR) were studied using logistic regression analysis.

Results Background of clinical profiles was as follows: median (range) age =71 (70-76) years, male/female =13/18, and median (range) HCV-RNA=260 (<5-3,800) KIU/mL. Out of 31, 16 patients (51.6%) had SVR by the intention-to-treat analysis. The SVR was significantly associated with the serum HCV RNA level. Logistic analysis showed that SVR occurred when HCV RNA level was <100 KIU/mL (p=0.020). Based on the difference of the serum HCV RNA level, the SVR rate was 81.8% (9/11) in patients with a serum HCV RNA level of <100 KIU/mL and 35.0% (7/20) in patients with a serum HCV RNA level of ≥100 KIU/mL.

Conclusion IFN-beta monotherapy of ≤24 week is a possible therapy selection for elderly patients of ≥70 years with type C hepatitis of genotype 2.

Key words: elderly patients, hepatitis C virus, genotype 2a or 2b, interferon monotherapy, sustained virological response

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Introduction

Current interferon (IFN) therapy for patients with chronic hepatitis C viral (HCV) infection has been directed at viral clearance. Recent studies have reported improvement of therapeutic efficacy when IFN is combined with ribavirin (1-6). However, IFN is expensive and has a number of serious side effects. The adverse events have a tendency to occur in elderly patients (7, 8). Therefore, in the case of elderly patients, the physician in charge often avoids IFN therapy because of IFN side effects. However, recently, the lifespan has been long in Japan. Thus, in the near future, a large number of patients with HCV will be >60 years of age. Also, HCV-related hepatocellular carcinoma (HCC) patients have been shown to become old with a peak around age 70 (9-11). When such aged patients with chronic abnormal ALT levels consult a doctor, the decision of whether or not to use therapy for chronic hepatitis is problematic. Moreover, when the use of treatment for chronic hepatitis C is decided for such aged patients, whether or not IFN therapy should be second problem.

A few studies have targeted IFN therapy and prolonged prognosis in elderly patients with chronic hepatitis C. Our

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investigation showed that the clearance of hepatitis C virus reduces the onset of HCC in elderly patients with HCV (12). Imai et al reported that IFN therapy reduces liver-related mortality in aged patients with chronic hepatitis C, especially in those exhibiting a biochemical response as well as a sustained virological response (13). Thus, in Japan, elderly patients with HCV are often treated with IFN.

In IFN therapy for chronic hepatitis C, several predictive factors of sustained virological response (SVR) to IFN have been identified, and these include short duration of disease, young age, absence of liver cirrhosis, genotype 2, low HCV-RNA levels, HCV and mutant type of nonstructual5A region (14-18). Thus, HCV patients with genotype 2 or low HCV-RNA levels might have the possibility of eradication of HCV RNA with a small dose or a short period of IFN. Now, there is also controversy about the indication and administration method of the IFN therapy in elderly patients with HCV.

Thus, in this study, we evaluated the efficacy of interferon (IFN)-beta monotherapy for type C patients of ≥70 years with genotype 2.

Abbreviation: ALT: alanine aminotransferase, AST: aspartate aminotransferase, CH: chronic hepatitis, HCV: hepatitis C virus, IFN: interferon, LC: liver cirrhosis, MU: million unit, SVR: sustained virological response

Materials and Methods

Patients

A total of 31 consecutive cirrhotic type C patients treated with IFN-beta for HCV RNA clearance at Toranomon Hospital in Tokyo, Japan between 2000 and 2007 were enrolled in this study. This study was a retrospective cohort study. Enrollment criteria were: ≥70 years; positive serum HCV RNA; genotype 2a or 2b; IFN-beta monotherapy; treatment period of ≤24 weeks. We excluded from the study all of the following patients: 1) those with concurrent hepatitis B virus (HBV); 2) with a history of IFN therapy; 3) leukocytes <3,000/mm^3, platelets <70,000/mm^3 and bilirubin >1.5 mg/mL before IFN therapy; 4) decompensated liver cirrhosis with ascites or encephalopathy.

IFN therapy

For the first IFN treatment regimen, the IFN treatment consisted of 3 to 6 million units (MU) of IFN-beta (Toray Industries or Daiichi Pharmaceutical Co., Tokyo, Japan). For the IFN treatment regimen, one group of 20 patients was given to receive IFN-beta intravenously every day for the first 2-8 weeks and then two or three times a week for the following 16-22 weeks (long-term group). Another group of 11 patients were treated with IFN by intravenous injection daily for 6-8 weeks (short-term group). The physician in charge primarily determined the method of IFN treatment and dose of IFN. We regarded sustained virological response (SVR) to therapy as clearance of HCV RNA by amplicor method (19) for more than 6 months after cessation of therapy. Our study was approved by the institutional ethics review board of our hospital. The physician in charge explained to each patient the purpose and method of the treatment as well as the potential adverse reactions, and informed consent for treatment was then obtained.

Blood testing

Blood samples were obtained just before IFN therapy and stored at -80℃. Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems, Branchburg, NJ, USA) (20).

On the other hand, serum HCV-RNA at 6 months after the termination of IFN therapy was analyzed by the qualitative PCR assay (19). The lower detection limit of the qualitative assay is 100 copies/mL. HCV genotype was examined by the PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (21).

Liver staging

Ideally, the severity of chronic liver disease should be determined histologically from the results of liver biopsy. Only 12 (38.7%) of 31 patients underwent peritoneoscopy or liver biopsy before the age of 70; the remaining 19 patients did not undergo histological assessment on the first visit owing to their advanced age. In these patients, liver staging was determined by calculation using the equation to discriminate chronic hepatitis (CH) and liver cirrhosis (LC) as described by Ikeda et al (22).

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test. Independent factors that might have influenced SVR were studied using multiple logistic regression analysis, and the following variables were evaluated as prognostic factors: sex, age, body mass index, HCV RNA level, HCV genotype 2a or 2b, liver staging, biochemical factors (AST, ALT), platelet count, total IFN dose, and IFN regimen. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Abbreviation: AST: aspartate aminotransferase

Results

Patients' characteristics

Table 1 shows the characteristics of the 31 patients who had received IFN-beta monotherapy. Clinical profiles were as follows: median (range) age =71 (70-76) years, male/female =13/18, median (range) HCV-RNA=260 (<5-3,800) KIU/mL, and CH/LC =19/12. All LC patients were catego-

Table 1. Clinical Characteristics before Interferon Therapy for Elderly Patients with Hepatitis C Virus of Genotype 2

Characteristics	(n=31)
Age (years old)	71(70-76)
Male/female	13/18
Body mass index	21.6 (17.3-25.4)
Complication of diabetes mellitus	0/31 (0%)
Complication of hypertension	4/31 (12.9%)
IFN therapy (short-regimen/long-regimen) *	11/20
Total dose of IFN (MU)	336 (12-624)
Liver Staging (CH/LC)	19/12
HCV load (KIU/mL)	260 (<5-3.800)
HCV genotype (2a/2b)	20/11
AST (IU/L)	55 (18-141)
ALT (IU/L)	65 (14-255)
Platelet (10^4/mm^3)	13.4 (7.3-21.6)
SVR	16/31 (51.6%)

ALT, alanine aminotransferase;
Data are expressed as number of patients or median (range)
* One group of 20 patients was given to receive IFN-beta intravenously every day for the first 2-8 weeks and then two or three times a week for the following 16-22 weeks (long-term group). Another group of 11 patients were treated with IFN by intravenous injection daily for 6-8 weeks (short-term group).

rized as Child-Pugh-Turcotte score class A.

Safety and tolerance in IFN group

Of the 31 patients originally included in this study, three discontinued IFN therapy due to owing to adverse events: that is, one patient each of nausea on the 3rd day after the initiation of IFN, general fatigue on the 7th day, and poor appetite at the 22nd week. On the other hand, for four patients the dose of the IFN therapy was reduced from 6 MU to 3 MU because of general fatigue and thrombocytopenia at 3-8 weeks after the initiation of IFN. Of these four patients one was in the short-term regimen and three in the long-term regimen. Thus, the median total dose was 336 MU (range, 12-624MU).

Efficacy of treatment

Out of 31 patients enrolled on the present study, 16 patients (51.6%) had SVR by the intention-to-treat analysis. The SVR was significantly associated with serum HCV RNA level. The patients with a HCV RNA level of <100 KIU/mL tended to have a high SVR compared to those with a HCV RNA level of ≥100 KIU/mL (p=0.020) (Table 2). Based on the difference of the serum HCV RNA level, the SVR rate was 81.8% (9/11) in patients with a serum HCV RNA level of <100 KIU/mL and 35.0% (7/20) in patients with a serum HCV RNA level of ≥100 KIU/mL. Serum HCV RNA at 4 week after the initiation of IFN could be determined in twenty-nine patients. The negativity rate of serum HCV RNA at 4 week after the initiation of IFN was 76.2% (16/21) in the SVR group and 0% (0/8) in the non-SVR group. Table 3 shows the differences in the clinical background between patients with SVR and those without SVR. The serum level of HCV RNA in patients with SVR was lower than that in patients without SVR. Table 4 shows the SVR rate based on the HCV load and IFN regimen. Table 5 shows the SVR rate based on the HCV load and the total dose of IFN. In patients with low virus load, the SVR rate in patients treated by the short-term regimen or a total dose of IFN of <350 MU was almost the same as that in patients treated by the long-term regimen or a total dose of ≥ 350 MU. On the other hand, in patients with high virus load, a high total dose has a tendency to enhance the SVR.

Discussion

The present study was limited by the fact that it was a non-randomized controlled trial. Another limitation of the study was that the number of patients was small. However, several findings from the present study have direct implications for the IFN treatment of elderly patients with genotype 2a or 2b.

First, about half of the patients of genotype 2 treated with IFN-beta monotherapy cleared HCV RNA. This result indicates that the IFN monotherapy is a possible selection of therapy for elderly patients with genotype 2. Second, the patients with HCV RNA level of <100 KIU/mL tend to have high SVR compared to those with a HCV RNA level of ≥ 100 KIU/mL. On the treatment regimen, the efficacy in the short-term regimen of IFN therapy was almost the same as that of the long-term regimen in patients with low-virus load. Moreover, the efficacy of the total dose of IFN of < 350 MU did not differ from that of a total dose of ≥350 MU in patients with a low virus load. These results indicate that in about 80% of elderly patients with a genotype 2 and serum HCV RNA level of <100 KIU/mL, HCV was eradicated by the 6- to 8-week regimen or total dose of IFN of < 350 MU. On the other hand, in patients with a high virus load, a high total dose might have a tendency to enhance the SVR.

Table 2. Predictive Factors for SVR in Interferon Therapy for Elderly Patients with Hepatitis C Virus of Genotype 2

Factor	Category	Odds ratio	95% CI	p value*
HCV RNA (KIU/mL)	<100 /≥100	1/8.36	1.40-49.88	.020
AST (IU/L)	≥38 /<38	1/0.57	0.81-4.01	.573
Age (years)	<75/ ≥75	1/0.75	0.14-4.10	.740
Platelet (10^4/mm^3)	≥15/<15	1/0.64	0.15-2.77	.553
Liver staging	(CH /LC)	1/0.90	0.21-3.85	.886
Sex	Female / Male	1/1.17	0.28-4.87	.883
ALT (IU/L)	≥50/<50	1/0.60	0.13-2.78	.521
Total dose of IFN (IU/L)	≥400/<400	1/0.67	0.16-2.77	.577
IFN regimen †	long / short	1/1.67	0.60-4.66	.330
HCV genotype	2b/2a	1/4.95	0.99-24.88	.052
Body mass index	<25/≥25	1/1.08	0.18-6.44	.930

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; CI, confidence interval; HCV, hepatitis C virus; IFN, interferon; LC, liver cirrhosis; SVR, sustained virological response;

*p value calculated by logistic regression analysis, Negativity rate of serum HCV RNA at 4week after the initiation of IFN was 76.2%(16/21) in the SVR group and 0%(0/8) in the non-SVR group.

† One group of 20 patients was given to receive IFN-beta intravenously every day for the first 2-8 weeks and then two or three times a week for the following 16-22 weeks (long-term group). Another group of 11 patients were treated with IFN by intravenous injection daily for 6-8 weeks (short-term group).

Table 3. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR

	SVR (n=16)	Non-SVR (n=15)	p value *
Age (years)	70 (70-76)	71 (70-76)	0.379
Sex (male/female)	8/9	6/9	0.735
Liver staging (CH/LC)	10/6	9/6	1.000
Body mass index	21.7(17.3-25.7)	20.3(18.8-25.9)	0.766
Total dose of IFN (MU)	336 (90-624)	336 (12-624)	0.545
IFN method (short term/long term) †	6/10	5/10	1.000
HCV genotype (2a/2b)	13/3	7/8	0.066
HCV-load (KIU/mL)	120 (<5-2300)	580 (33-3800)	0.041
HCV RNA at 4 week (-/+)	16/0	7/8	<0.001
AST (IU/L)	59 (25-141)	54 (18-99)	0.313
ALT (IU/L)	65 (17-255)	59 (14-148)	0.667
Platelet (10^4/mm^3)	14.9 (7.3-21.6)	14.1 (10.4-22.0)	0.626

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; HCV, hepatitis C virus; IFN, interferon; LC, liver cirrhosis; MU, million unit; SVR, sustained virologic response;

Data are expressed as number of patients or median (range),

*p value calculated by the Mann-Whitney U test

† One group of 20 patients was given to receive IFN-beta intravenously every day for the first 2-8 weeks and then two or three times a week for the following 16-22 weeks (long-term group). Another group of 11 patients were treated with IFN by intravenous injection daily for 6-8 weeks (short-term group).

Table 4. The SVR Rate Based on the HCV Load and IFN Regimen

HCV load (KIU/mL)	IFN regimen*		Total
	Short-term (6-8 weeks)	Long-term (24 weeks)	
Low-virus load (<100)	83.3% (5/6)	80.0% (4/5)	81.8% (9/11)
High-virus load (≥100)	20.0% (1/5)	40.0% (6/15)	35.0% (7/20)
Total	54.5% (6/11)	50.0% (10/20)	51.6% (16/31)

HCV, hepatitis C virus; IFN, interferon.

* One group of 20 patients was given to receive IFN-beta intravenously every day for the first 2-8 weeks and then two or three times a week for the following 16-22 weeks (long-term group). Another group of 11 patients were treated with IFN by intravenous injection daily for 6-8 weeks (short-term group).

Table 5. The SVR Rate Based on the HCV Load and a Total Dose of IFN

HCV load (KIU/mL)	A total dose of IFN (million units)		Total
	<350	≥350	
Low-virus load (<100)	75% (6/8)	100% (3/3)	81.8% (9/11)
High-virus load (≥100)	20% (2/9)	45.4% (5/11)	35.0% (7/20)
Total	47.1% (8/17)	57.1% (8/14)	51.6% (16/31)

HCV, hepatitis C virus; IFN, interferon.

Regarding the side effects of IFN, three patients withdrew the treatment due to IFN-related side effect. Moreover, four patients had to reduce the IFN dose due to IFN side effects. For IFN therapy for elderly patients, the physician in charge should check the clinical findings compared to young patients.

At present, the combined IFN and ribavirin therapy is a standard therapy for chronic hepatitis C patients with a high load of HCV-RNA. However, prolonged combination therapy of IFN and ribavirin is associated with various side effects. If the total dose of IFN is decreased and the period of IFN therapy is short, it would be desirable from two points: cost and side effect.

IFN-beta should be given intravenously. The intravenous injection is not convenient for treatment compared to intramuscular or subcutaneous injection. However, IFN-beta-related side effects are mild and few compared to combination therapy of IFN-alpha. Katamura et al has reported that IFN-beta-induced mental disorders are milder than those induced by PEG-IFN (23). The present study indicates that patients ≥70 years old tolerate IFN-beta.

Fortunately, in patients with genotype 2 and low virus load, HCV RNA tends to be eradicated with a small dose of IFN (24-27). The present study indicates that in elderly patients of ≥70 years with a low HCV-RNA, HCV RNA can be eradicated with a low dose of IFN.

Conclusion

The present study indicates that IFN-beta monotherapy of ≤24 weeks is a possible selection of therapy for elderly patients of ≥70 years old with type C hepatitis of genotype 2.

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Losartan Reduces the Onset of Type 2 Diabetes in Hypertensive Japanese Patients With Chronic Hepatitis C

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The aim of this retrospective cohort study is to assess the cumulative development incidence and predictive factors for type 2 diabetes (T2DM) in HCV positive and hypertensive patients treated with losartan. Eighty Japanese patients were given 50 mg of losartan per day after diagnosis of hypertension (losartan group). Another 160 treated with spironolactone were selected as control (spironolactone group). Patients in spironolactone group were matched 1:2 with losartan group for age and sex. The mean observation period was 5.2 years in losartan group and 5.4 years in spironolactone group. An overnight (12 hr) fasting blood sample or a casual blood sample was taken for routine analyses during follow-up. The primary goal is the onset of T2DM. Evaluation was performed by using the Kaplan–Meier method and the cox proportional hazards analysis. Three patients in losartan group and 20 in spironolactone group developed T2DM. The 5th year cumulative appearance rates of T2DM were 5.4% in losartan group and 14.4% in spironolactone group. Multivariate cox proportional hazards analysis showed that T2DM development after the initiation of anti-hypertensive drugs occurred when anti-hypertensive drug was spironolactone (hazard ratio: 6.10; 95% confidence interval = 1.78–20.84; $P = 0.004$), histological staging was advanced (hazard ratio: 4.31; 95% confidence interval = 1.94–9.60; $P < 0.001$), fatty liver was present (hazard ratio: 3.28; 95% confidence interval = 1.47–7.27; $P = 0.004$), and patient had pre-diabetes (hazard ratio: 2.47; 95% confidence interval = 1.08–5.63; $P = 0.032$). Our results indicate losartan causes about 60% reduction of the onset of T2DM compared to patients treated with spironolactone. ***J. Med. Virol. 81:1584–1590, 2009.***

KEY WORDS: hepatitis C virus; hypertension; losartan; type 2 diabetes mellitus; a retrospective cohort study

INTRODUCTION

Hepatitis C virus (HCV) is one of the more common causes of chronic liver disease in world. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis and/or hepatocellular carcinoma (HCC) over a period of 10–30 years [Kiyosawa and Furuta, 1991; Alter et al., 1992; Ikeda et al., 1993; Tsukuma et al., 1993]. Additionally, data supporting a link between Type 2 diabetes mellitus (T2DM) and chronic hepatitis C

Abbreviations used: ALT, alanine aminotransferase; normal range = 11–36; AST, aspartate aminotransferase; normal range = 6–34; CI, confidence interval; FPG, fasting plasma glucose; HCV, hepatitis C virus; HR, hazard ratio.

Specific author contributions: Yasuji Arase: design, data collection, data analysis, manuscript development and oversight; Fumitaka Suzuki: design, data collection, data analysis, manuscript development; Yoshiyuki Suzuki: data collection; Norio Akuta: data collection; Masahiro Kobayashi: data collection; Yusuke Kawamura: data collection; Hiromi Yatsuji: data collection; Hitomi Sezaki: data collection; Tetsuya Hosaka: data collection; Miharu Hirakawa data collection: Kenji Ikeda: data collection; Hiromitsu Kumada: design, data collection, data analysis, manuscript development and oversight; Tetsuro Kobayashi: manuscript development and oversight.

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infection have been reported [Arao et al., 2003; Mehta et al., 2003; Romero-Gómez et al., 2008; Imazeki et al., 2008; Arase et al., 2009]. Recently, hypertension increased in chronic liver disease with increase of elderly patients in Japan. Administration of losartan has been proven to be useful for the treatment of hypertension [Dahlöf et al., 2002; Lindholm et al., 2002]. Some previous studies have presented conflicting results with some suggesting that angiotensin receptor antagonist improves insulin sensitivity and exert beneficial effects on glucose and lipid metabolism [Iimura et al., 1995; Yusuf et al., 2000; Ando and Fujita, 2006]. Whereas others found that losartan did not influence insulin sensitivity [Fogari et al., 1998]. These discrepancies might depend on factors such as race, age, stage of hypertension, structural vascular changes in precapillary arteries. However, in any case, there is little information on the yearly cumulative incidence and risk factors on the development rate of T2DM in hypertensive patients with type C chronic liver disease during the prolonged follow-up.

In Toranomon Hospital (Tokyo, Japan), the authors evaluate a large number of patients with HCV-related hepatitis, and often find hypertension and T2DM. With this background in mind, the cohort study was initiated to investigate the cumulative incidence and risk factors of T2DM after prolonged follow up in HCV-infected and hypertensive patients treated with antihypertensive drugs. The strength of the current study is the long-term follow-up of patients.

METHODS

Patients

The number of patients who were diagnosed with chronic HCV infection between April 1998 and March 2007 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan was 5,400. Out of these, 890 were given antihypertensive therapy after confirmation of blood pressure $\geq$140 mm Hg systolic and/or $\geq$90 mm Hg diastolic on at least 3 visits and absence of secondary causes of hypertension, previous cardiovascular disease and stroke, and life threatening conditions. Blood pressure was measured by a physician with a mercury sphygmomanometer, with subjects sitting and relaxed for at least 10 min. Inclusion criteria were as follows: (1) antihypertensive therapy by losartan; (2) 45–75 years old; (3) no evidence of diabetes mellitus for 3 months before the initiation of anti-hypertensive therapy: a plasma glucose concentration of <126 mg per deciliter (6.9 mmol/L) in the fasting state, <200 mg per deciliter (11.0 mmol/L) in casual state and/or 2 hr after a 75-g oral glucose load; (4) features of chronic hepatitis or cirrhosis diagnosed by clinical features, laboratory tests, ultrasonographic findings, or histological findings; (5) positive for anti-HCV and HCV-RNA; (6) negative for hepatitis B surface antigens (HBsAg), antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay or spot hybridization; (7) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; (8) no underlying systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis. Patients with either of the following criteria were excluded from the study: (1) they were taking medicines known to alter glucose tolerance, (2) decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites (3) they had illnesses that could seriously reduce their life expectancy or their ability to participate in the trial. Eighty patients were selected as losartan group. Patients were classified as having normal glucose group or pre-diabetes group base to the fasting plasma glucose (FPG), casual plasma glucose, or 2-hr plasma glucose: (1) normal glucose group was regarded as having FPG of <100 mg/dl, casual plasma glucose of <140 mg/dl, and/or 2-hr plasma glucose of <140 mg/dl, (2) pre-diabetes group was regarded as having FPG of 100–125 mg/dl, casual plasma glucose of 140–200 mg/dl, and/or 2-hr plasma glucose of 140–200 mg/dl [Genuth et al., 2003] The patients in the losartan-group received 50 mg of losartan orally once a day.

In the same period, 382 hypertensive patients with HCV positive chronic liver disease were treated with spironolactone. The 321 patients were applied with seven inclusion criteria and three exclusion criteria described in losartan group. One hundred sixty subjects in spironolactone group were selected from these 321 patients by matching 1:2 with losartan group for age and sex. Thus, differences of the cumulative appearance rate of T2DM in the losartan group and spironolactone group were compared. The patients in spironolactone group were treated with spironolactone at a dose of 25 or 50 mg once daily. Next, predictive factors for T2DM in both groups were assessed. The physicians in charge explained the purpose and method of antihypertensive treatment to each patient and/or patients' family, who gave their informed consent for the treatment. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study had been approved by Institutional Review Board of Toranomon hospital.

Outcome Measures

The primary outcome was T2DM, diagnosed by the use of the 2003 criteria of the American Diabetes Association [Genuth et al., 2003]. That is, the criteria for the diagnosis of diabetes mellitus include: (a) casual plasma glucose $\geq$200 mg/dl; (b) FPG $\geq$126 mg/dl; (c) 2 hr post-glucose (oral glucose tolerance test) $\geq$200 mg/dl. At the same time, clinical records of cardiovascular events (angina pectoris, heart infarction) and stroke (cerebral infarction, cerebral bleeding) were examined.

Laboratory Inveastigation

Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche, Tokyo, Japan). HBsAg was

tested by radioimmunoassay (Abbott Laboratories, Detroit, MI). The used serum samples were stored −80°C at the first consultation. Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA. Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (in kg)/height (in m^2)

Evaluation of Liver Cirrhosis and Fatty Liver

Status of liver cirrhosis was mainly determined on the basis of peritoneoscopy and/or liver biopsy. The 183 out of 260 were diagnosed by peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas. Baseline liver histology of chronic hepatitis was classified according to the extent of fibrosis, into four stages in progression order: stage 1, periportal expansion; stage 2, portoportal septa; stage 3, portocentral linkage or bridging fibrosis; stage 4, liver cirrhosis [Desmet et al., 1994]. Remaining patients were diagnosed by clinical features, laboratory tests, and ultrasonographic findings.

Diagnosis of fatty liver was based on the presence of an ultrasonographic pattern consistent with bright liver (brightness and posterior attenuation) with stronger echoes in the hepatic parenchyma than in the renal or spleen parenchyma, vessel blurring, and narrowing of the lumen of the hepatic veins. US was performed with a high-resolution, real-time scanner (model SSD-2000; Aloka Co., Ltd, Tokyo Japan. Mode Logic-700 MR; GE-Yokokawa Medical Systems, Tokyo, Japan).

Follow-Up

The starting time of follow-up was the initiation of antihypertensive therapy. After that, patients were followed up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check up. An overnight (12 hr) fasting blood sample or a casual blood sample was taken for routine analyses. These included transaminase activities, total cholesterol, platelet counts, and serum HCV RNA level. Twenty-one patients were lost to follow-up. Because the appearance of T2DM and death was not identified in these 21 patients, they considered as censored data in statistical analysis [Fleming et al., 1984]. Patients treated with antiviral agents were regarded as withdrawals at the time of starting the treatment of antiviral agents. Moreover, patients with change or addition of hypertensive drugs were regarded as withdrawals at the time of change or addition of hypertensive drugs. Finally, patients with decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites were regarded as withdrawals.

Statistical Analysis

The cumulative appearance rate of T2DM was calculated from the initiation of hypertensive drugs using the Kaplan–Meier method. Differences in the development of T2DM were tested using the log rank test. Independent factors associated with the incidence rate of T2DM were analyzed by the Cox proportional hazard model. The following eleven variables were analyzed for potential covariates for incidence of T2DM after the time of initiation of hypertensive drugs at our hospital: age, sex, hepatic staging (chronic hepatitis or liver cirrhosis), BMI, glucose level, aspartate aminotransferase (AST), alanine aminotransferase (ALT) level, triglyceride level, total cholesterol level, and treatment. A P-value of <0.05 was considered significant. Data analysis was performed using the computer program SPSS package (SPSS 11.5 for Windows, SPSS, Chicago, IL).

RESULTS

Patients' Characteristics

Table I shows the characteristics of the 240 HCV positive and hypertensive patients enrolled in the present study. There were no significant differences in clinical profiles between the losartan and spironolactone group. The mean observation period was 5.2 years in losartan group and 5.4 years in spironolactone group. On side effects, two patients treated with losartan had episodes of dizziness. In spironolactone group, four patients had gynecomastia and two patients had dizziness. However, they could continue without stopping the antihypertensive therapy using losartan or spironolactone.

Incidence of T2DM in Hypertensive Patients With HCV

A total of 25 subjects (15 men and 10 women) developed T2DM during the observation period. Three patients in losartan group and 22 in spironolactone group developed T2DM. The 5th year cumulative appearance rates of T2DM were 5.9% in losartan group and 14.0% in spironolactone group (Fig. 1). Multivariate cox proportional hazards analysis showed that development of T2DM when anti-hypertensive drug was spironolactone (hazard ratio: 6.10; 95% confidence interval = 1.78–20.84; $P = 0.004$), histological staging was advanced (hazard ratio: 4.31; 95% confidence interval = 1.94–9.60; $P < 0.001$), fatty liver was present (hazard ratio: 3.28; 95% confidence interval = 1.47–7.27; $P = 0.004$), and patient had pre-diabetes (hazard ratio: 2.47; 95% confidence interval = 1.08–5.63; $P = 0.032$) (Table II). Our results indicate losartan causes about 60% reduction of the risk of T2DM development compared to spironolactone.

Figure 2 shows the impact of reduction due to administration of losartan on the incidence of T2DM in patients with liver cirrhosis, or pre-diabetes, or fatty liver. When patients with liver cirrhosis are treated with

TABLE I. Clinical Characteristics at the Time of Initiation of Anti-Hypertensive Drug

	Total	Losartan group	Spironolactone group	*P*-value
N	240	80	160	
Age (years)	65.2 ± 8.2	65.2 ± 8.0	65.2 ± 8.2	1.0
Sex (male/female)	120/120	40/40	80/80	1.0
Blood pressure				
Systolic (mm Hg)	161.8 ± 13.0	163.0 ± 14.1	160.9 ± 12.3	0.366
Diastolic (mm Hg)	94.3 ± 7.4	95.1 ± 8.2	93.9 ± 6.9	0.596
Staging (chronic hepatitis/liver cirrhosis)	194/46	64/16	130/30	0.863
F1/F2/F3/F4[a]	51/79/22/40	14/31/7/14	37/48/15/24	0.251
Fatty liver (+/−)[b]	48/192	14/66	34/126	0.608
BMI	23.7 ± 4.5	23.2 ± 3.5	23.9 ± 5.2	0.250
AST (IU/L)	77.5 ± 60.3	73.7 ± 49.2	78.8 ± 63.2	0.297
ALT (IU/L)	108.6 ± 99.8	108.8 ± 101.0	106.7 ± 94.2	0.604
Albumin (g/dl)	4.2 ± 0.4	4.2 ± 0.4	4.2 ± 0.5	0.717
γ-GTP (IU/L)	59.3 ± 58.5	58.2 ± 59.3	59.6 ± 60.8	0.862
Platelet count ($\times 10^4/mm^3$)	16.9 ± 5.6	15.8 ± 6.3	17.2 ± 5.4	0.089
Glucose level (prediabetes/normal)	42/198	15/65	27/133	0.722
T cholesterol (mg/dl)	172.8 ± 33.4	176.2 ± 53.5	172.5 ± 32.5	0.965
Triglyceride (mg/dl)	104.5 ± 47.1	97.0 ± 28.9	105.2 ± 48.9	0.063

Data are number of patients or mean ± standard deviation, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γ-GTP, γ-glutamyl transpeptidase.
[a]Histological diagnosis of the liver.
[b]Diagnosis of fatty liver by the ultrasonography.

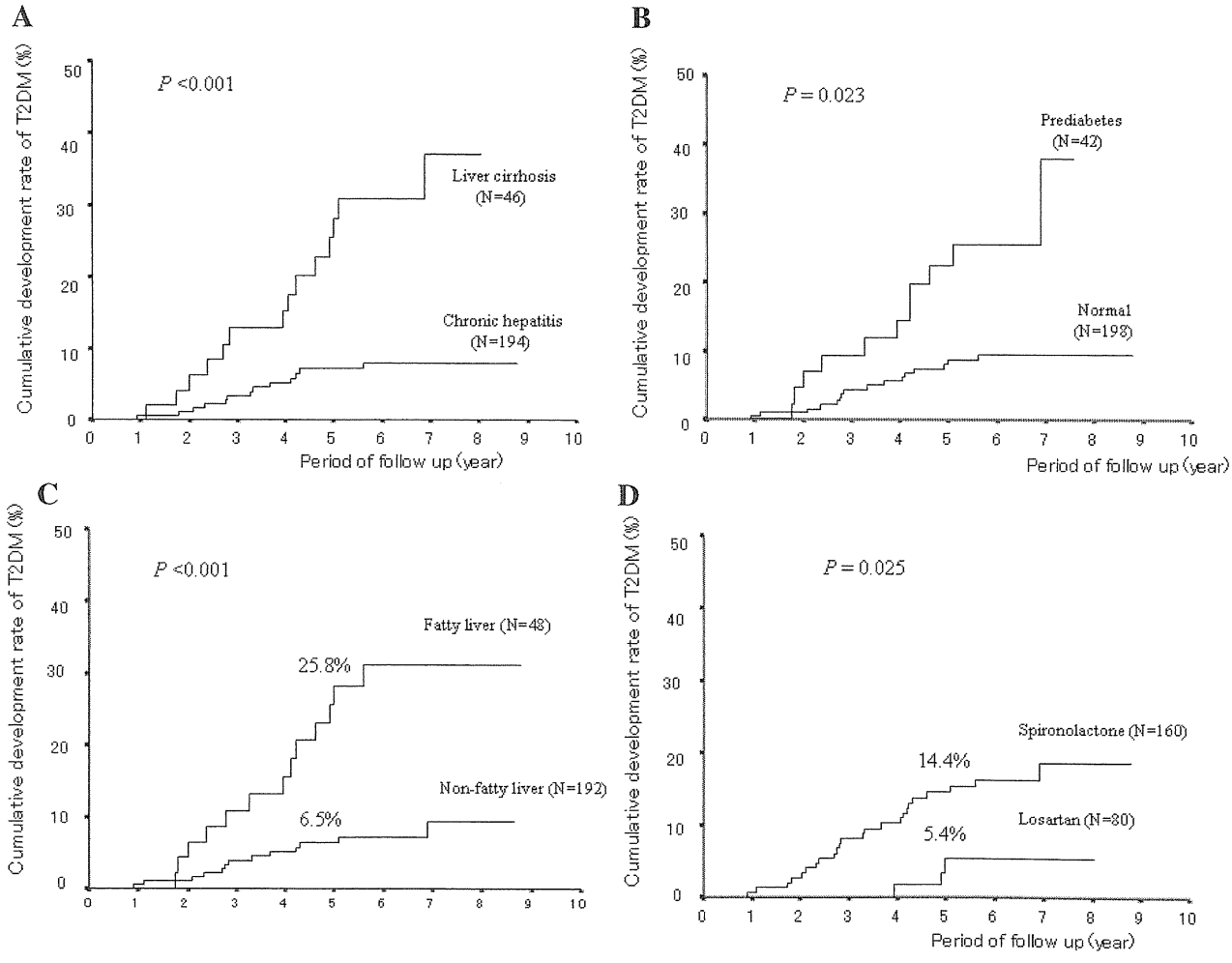

Fig. 1. Cumulative development rate of T2DM in patients treated with interferon. **Panel A**: Cumulative development rate of T2DM based on difference of hepatic fibrosis; (**Panel B**), cumulative development rate of T2DM based on the difference of glucose level; (**Panel C**), cumulative development rate of T2DM based on the difference of fatty liver; (**Panel D**), cumulative development rate of T2DM based on the difference of anti-hypertensive drugs.

TABLE II. Predictive Factors for T2DM Development

Variables	N	Univariate analysis		Cox-regression	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Age (years, ≥65/<65)	121/119	2.28 (1.02–5.07)	0.044		
Sex (female/male)	120/120	0.60 (0.28–1.28)	0.184		
BMI (≥25/<25)	60/180	2.42 (1.091–5.33)	0.028		
Maximum BMI (≥25/<25)	55/141	1.76 (0.76–4.06)	0.190		
Fatty liver (+/−)	48/192	4.35 (2.01–5.07)	<0.001	3.28 (1.47–7.27)	0.004
Genotype (1/2)	162/45	0.91 (0.39–2.88)	0.905		
ALT (IU/L, ≥50/<50)	151/89	1.14 (0.38–3.42)	0.822		
Glucose level (prediabetes/normal)	42/198	2.93 (1.33–6.48)	0.022	2.47 (1.08–5.63)	0.032
Triglyceride (mg/dl, ≥150/<150)	34/135	1.85 (0.83–5.98)	0.095		
Cholesterol (mg/dl, <220/≥220)	172/40	0.54 (0.06–5.16)	0.590		
Staging (liver cirrhosis/chronic hepatitis)	46/194	4.25 (1.97–9.18)	0.023	4.31 (1.94–9.60)	<0.001
AST (IU/L, ≥38/<38)	168/72	0.96 (0.32–2.881)	0.942		
Treatment (spironolactone/losartan)	160/80	3.94 (1.19–13.15)	0.025	6.10 (1.78–20.84)	0.004

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HCV, hepatitis C virus; HR, hazards ratio.

losartan, losartan could statistically reduce the onset of T2DM compared to those with spironolactone.

Incidence of Cardio Vascular Disease or Stroke in Patients With HCV

A total of eight subjects (five men and three women) developed vascular events during the observation period. In losartan group, two patients developed stroke. In spironolactone group, three patients developed cardiovascular disease and another three patients developed stroke.

Figure 3 shows the impact of reduction due to difference of antihypertensive drugs on the incidence of cardiovascular disease or stroke in two groups. There was little difference on losartan group and spironolactone group.

DISCUSSION

We have described the development incidence of T2DM after the initiation of antihypertensive therapy in HCV positive patients treated with antihypertensive drugs in the present study. The present study was limited by a retrospective cohort trial. About the sample size in losartan and spironolactone group, the number of the patients enrolled in the present study was sufficient to detect hazards ratios of about threefold with 80% power at the 5% level of significance. The strength of the present study is a long-term follow-up in the patients included.

The present study shows several findings with regard to development of T2DM after the initiation of losartan or spironolactone for HCV positive and hypertensive patients. First, the T2DM development rate in losartan group was lower than that in spironolactone group. The administration of losartan caused about 60% reduction in the onset of T2DM in the course of follow-up. What losartan enhances the insulin sensitivity has been reported by some authors [Iimura et al., 1995; Ando and Fujita, 2006; Alderman, 2008]. However, protection of T2DM development by losartan in the present study was effective compared to that based on Dahlöf's report [Dahlöf et al., 2002]. This discrepancy is thought to be due to difference of race and HCV infection. Our previous study shows that clearance of HCV causes a two-thirds reduction of the onset of T2DM in hepatitis C virus positive patients treated with interferon [Arase et al., 2009]. This means that patients with HCV have a high tendency of the onset of T2DM. Moreover, although the prevalence of T2DM is increasing dramatically in USA, increases in newly developed and developing countries in Asia have been ever greater [Yoon et al., 2007]. Thus, Asian patients with HCV are thought to have high risk of T2DM. Anti-diabetic effect of losartan may also enhance in patients with high risk of T2DM.

Though the role of losartan in preventing development of DM remains speculative, the following possible mechanism have been reported, (1) losartan elevates the serum level of adiponectin that improves insulin sensitivity [Clasen et al., 2005]; (2) losartan enhance the insulin-like growth factor (IGF)-1 that plays a protective role in the development of glucose intolerance [Zandbergen et al., 2006].

Second, in addition to administration of spironolactone, the present study suggests that aging, progression of hepatic staging, pre-diabetes enhanced the onset of T2DM in HCV patients treated with anthypertensive drugs.

The present study indicates that losartan reduce the onset of T2DM in Japanese patients with HCV. Our retrospective study suggests that the annual incidence of T2DM among patients with HCV was determined to be 1.0–1.1% in losartan group and 2.8–3.0% in spironolactone group. Moreover, several lines of evidence have shown that angiotensin receptor antagonist can have a beneficial role in the early stages of hepatic fibrosis of patients with hepatitis C [Terui et al., 2002]. Thus, when physicians regarding the daily management of patients with virus hepatitis give antihypertensive therapy for HCV patients, they should

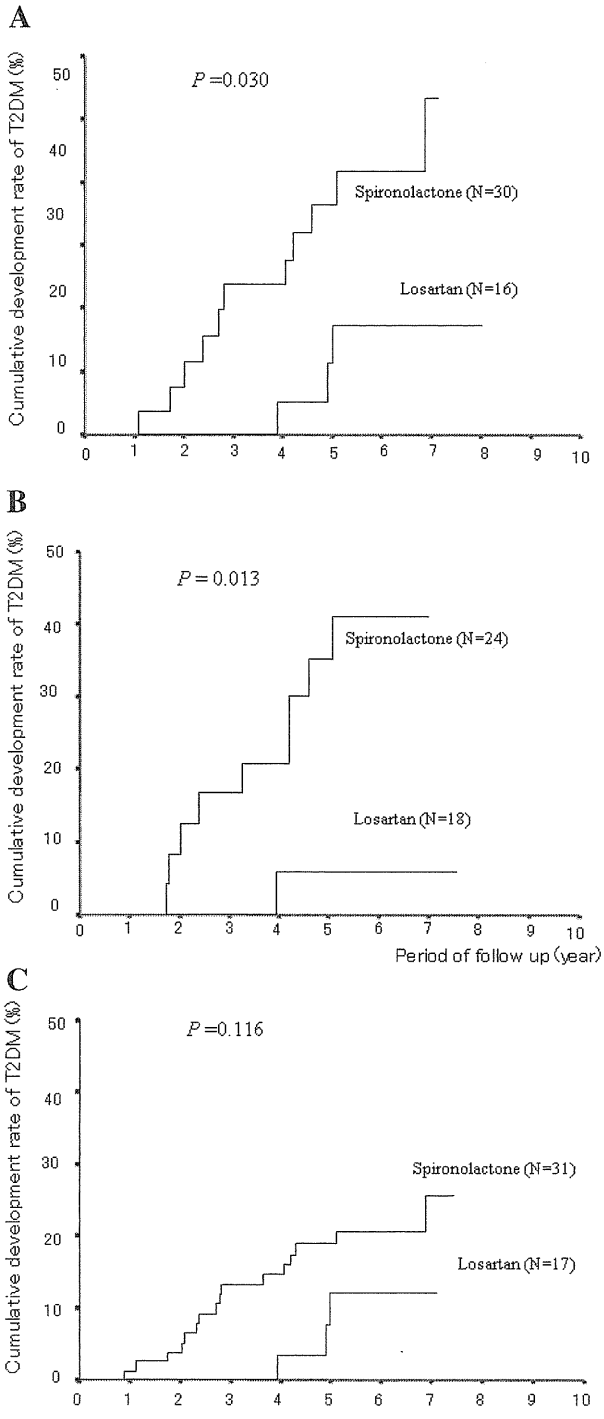

Fig. 2. Cumulative development rate of T2DM in patients with losartan or spironolactone. **Panel A**: Cumulative development rate of T2DM based on the difference of anti-hypertensive drugs in patients with liver cirrhosis; (**Panel B**), cumulative development rate of T2DM based on the difference of anti-hypertensive drugs in patients with pre-diabetes; (**Panel C**), cumulative development rate of T2DM based on the difference of anti-hypertensive drugs in patients with fatty liver.

consider the indication of losartan for protecting the onset of T2DM and progression of liver fibrosis.

In conclusion, our results indicate losartan causes about 60% reduction of the risk of T2DM development in HCV positive, hypertensive, Japanese patients.

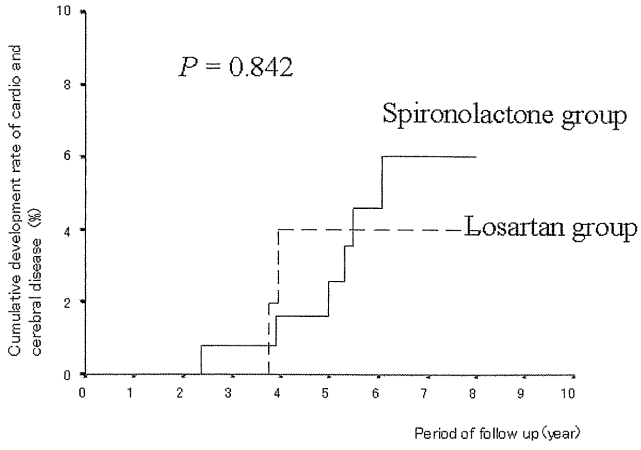

Fig. 3. Cumulative development rate of cardiovascular disease and stroke based on the difference of anti-hypertensive drug.

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