Original Article

Interleukin-18/Interleukin-10 ratio is an independent predictor of SVR patients treated with IFN-based therapy

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BACKGROUND/AIM: Nearly 20 % of patients with genotype 2 chronic hepatitis C (CHC) do not achieve a sustained virologic response (SVR), even if pegylated-IFN and ribavirin are administered for 24 weeks. The aim of this study was to determine the factors predicting the efficacy of interferon (IFN)-based therapy for patients infected with hepatitis C virus (HCV) genotype 2.

PATIENTS AND METHODS: Eighty-seven adults with CHC (M/F: 57/30) due to infection with HCV genotype 2a or 2b were studied. Thirty-six patients were treated with IFN-alpha alone, 30 patients received consensus IFN alone, and 21 were given IFN-alpha 2b plus ribavirin. In all three regimens, IFN was administered daily for 2 weeks, followed by the same dose thrice weekly for a median of 22 weeks (range: 10-46 weeks). A SVR was defined as undetectable (< 50 IU/ml) serum HCV- RNA at 24-week follow-up. Serum cytokines and standard liver function tests were measured before starting therapy. We retrospectively investigated the pretreatment parameters influencing SVR by logistic regression analysis.

RESULTS: SVR was achieved in 63 patients. The log-10 transformed serum level of HCV-RNA (log HCV-RNA) and log IL-18/IL-10 were factors with an independent influence on SVR on multivariate regression analysis.

CONCLUSION: This study provided the first evidence that the baseline serum IL-18/IL-10 ratio levels is an independent prognostic indicator for SVR in patients with CHC genotype 2.

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Key words chronic hepatitis C, IFN therapy, ribavirin, cytokines, predictor

Introduction

Chronic hepatitis C virus (HCV) infection is associated with an insidious and progressive form of liver disease that may eventually lead to cirrhosis or hepatocellular carcinoma[1,2]. Therefore, there is a great need to eliminate persistent HCV infection. The HCV genotype and baseline serum HCV-RNA load have the

greatest influence on the response to antiviral therapy in patients with chronic hepatitis C (CHC)[3]. Patients infected with HCV genotype 2 have a substantially better response rate to current antiviral therapies than those infected with genotype 1. In fact, when CHC patients with HCV genotype 2 are treated with a combination of glycosylated (pegylated) -interferon (PEG-IFN) -alpha plus ribavirin for 24 weeks, which is currently the standard therapy, a sustained virologic response (SVR) is achieved in approximately 80 %[4]. However, nearly 20 % of patients do not achieve SVR, even with this gold standard treatment.

IFN alone or combined with ribavirin is believed to work via modulation of the host immune response, rather than directly acting as an antiviral agent[5]. Some interleukins (IL), such as IL-10[6-8] or IL-18[9,10] were reported to be related with efficacy of IFN-based regimens.

Determining the factors that predict cure (both viral factors and host characteristics) has become important in CHC patients receiving PEG-IFN plus ribavirin therapy, suggesting that it might be useful to review the results of various non PEG-IFN-based regimens.

Since 2002, IFN regimens were stratified according to serum HCV-RNA level, such as IFN-alpha alone, consensus IFN (C-IFN) alone, or IFN-alpha combined with ribavirin (R+IFN). Between 1997 and 2001, IFN-alpha alone was used to treat CHC patients regardless their serum HCV-RNA level.

The goal of this study was to determine the pretreatment viral and host parameters associated with SVR in order to better define the efficacy of PEG- IFN plus ribavirin.

Patients And Methods

1)Patients

Between 1997 and 2007, 87 consecutive patients with CHC were studied. All of them were confirmed to be positive for serum anti-HCV antibodies by a third-generation ELISA (Ortho Diagnostic System, Tokyo, Japan) and they were also positive for serum HCV-RNA by the qualitative Amplicor HCV monitor Ver. 2.0 test (Roche Molecular Systems, Inc., Pleasanton, CA, USA). The enrollment criteria included an age between 21 and 72 years, a baseline serum HCV-RNA level measured by the quantitative Amplicor HCV monitor Ver 2.0 test (Roche) between 0.5 (the lower limit of this test) and 850 KIU (the upper limit), and elevation of ALT for at least 6 months. According to the serological genotyping assay reported by Tanaka et al.[11], all patients were infected with serotype 2 (genotypes 2a or 2b). Patients were excluded if they were positive for hepatitis B surface antigen (HBs-Ag) or antinuclear antibodies.

Eighty-five of the 87 patients underwent percutaneous liver biopsy under ultrasound guidance before treatment. Biopsy specimens were graded according to the Histology Activity Index (HAI) of Knodell et al.[12], and were divided into three grades according to the HAI score (Grade 1, HAI score of 1-3; Grade 2, HAI score of 4-8; Grade 3, HAI score of 9 or more)[13]. Biopsy specimens were also divided into four stages (1-4) based on the fibrosis score of Desmet[13], as shown in Table 1. The three treatment groups were comparable with regard to age, sex, and the baseline levels of AST, ALT, peripheral blood leukocyte count, platelet, and prothrombin time, as well as and pathological findings. Only the HCV-RNA load quantified by RT-PCR (Amplicor Ver 2.0) and the history of IFN therapy showed differences (Table 1).

This study was performed in accordance with the internationally accepted ethical standards for human experimentation. The purpose of the study and the protocol were explained to all of the patients and informed consent was obtained from each subject.

2)IFN-based regimens

Since 2002, we have mainly chosen IFN-alpha alone for CHC patients with genotype 2, and a low viral load (a baseline serum HCV-RNA level of than 100 KIU/ml), C-IFN alone for a moderate viral load (a baseline serum HCV-RNA level from 101 to less than 500 KIU/ml), and R+IFN for patients with a high viral load (a baseline serum HCV-RNA level of 501 KIU/ml or more). Between 1997 and 2001, we used IFN-alpha alone, because this was only approved in Japan at that time.

Thirty-six patients were treated with 5-10 MU of natural IFN-alpha alone (OIF®; Ootsuka Pharmaceutical Co. Ltd., Tokyo, Japan), 30 patients received 12-18 MU of C-IFN alone (Adovaferon®; Astellas Pharma Inc, Tokyo, Japan), and 21 patients were received R+IFN, which consists of 6 MU of IFN-alpha 2b (Intron A®; Schering Plough Pharmaceutical Co. Ltd., Tokyo, Japan) combined with 400-800 mg/day of ribavirin (Schering Plough) (Table 1).

All IFNs were administered daily for 2 weeks, followed by the same dose thrice weekly for a median of 22 weeks (range: 10-46 weeks) after informed consent was obtained. IFN therapy was completed without IFN dose reduction in all patients, but some patients in the combined therapy group needed slight adjustment of the ribavirin dose because their hemoglobin decreased during treatment.

3)Study Design

Serum HCV-RNA was measured by the quantitative Amplicor HCV monitor Ver. 2.0 test (Roche) before IFN therapy and by the qualitative Amplicor HCV monitor Ver. 2.0 test (Roche) at

Table 1 Background of the 3 groups

	IFN-alpha alone	C-IFN alone	R+ IFN	
No. of patients (n=)	36	30	21	
Age (y.o.)	Median: 49 (range: 32 - 65)	Median: 53 (range: 40 - 65)	Median: 50 (range: 38 - 68)	NS #
Sex (M/F)	27 / 9	18 / 12	12 / 9	NS ※
Laboratory data				
HCV-RNA (KIU/mL)	Median: 45 (range: 6 - 440)	Median: 160 (range: 68 - 445)	Median: 390 (range: 167 - 613)	P<0.01 #
AST (IU/L)	Median: 68 (range: 32 - 147)	Median: 63 (range: 30 - 156)	Median: 68 (range: 33 - 141)	NS #
ALT (IU/L)	Median: 108 (range: 39 - 236)	Median: 84 (range: 35 - 243)	Median: 71 (range: 23 - 267)	NS #
WBC (10^3/mm^3)	Median: 5.2 (range: 2.8 – 6.7)	Median: 5.2 (range: 3.3 – 7.2)	Median: 5.3 (range: 4.1 – 7.8)	NS #
Platelets (10^4/mm^3)	Median: 16.7 (range:9.7- 25.2)	Median: 14.7 (range:7.6 - 23.8)	Median: 16.2 (range:9.1- 23.6)	NS #
Prothrombin time (%)	Median: 92 (range: 76 - 107)	Median: 90 (range: 70 - 107)	Median: 94 (range: 82 - 100)	NS #
Previous IFN therapy No / Yes	35 / 1	25 / 5	12 / 9	P<0.01 ※
Pathological findings				
Activity (1 / 2 / 3 / none)	**8 / 24 / 3 / 1**	**10 / 14 / 6 / 0**	**5 / 11 / 4 / 1**	NS ※
Fibrosis (1 / 2 / 3 / 4 / none)	**15 / 9 / 8 / 3 / 1**	**13 / 6 / 7 / 4 / 0**	**5 / 5 / 6 / 4 / 1**	NS ※

Range represents 10^{th} to 90^{th} percentile.
NS: No significant difference. #: Tukey's test. ※: χ^2 test.

24 weeks after the end of treatment. When serum HCV-RNA was undetectable by the qualitative Amplicor HCV monitor Ver. 2.0 test at 24 weeks after finishing therapy, this was defined as a SVR. When serum HCV-RNA was detected by this test within 24 weeks of finishing therapy, the patient was classified as a non-responder (NR). The overall SVR rate was calculated and so was the rate for each treatment.

To investigate prognostic variables for SVR, we retrospectively analyzed various pretreatment parameters of the patients by logistic regression analysis.

4)Parameters

Before starting IFN therapy, a blood sample was collected from each patient in the early morning. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cholinesterase (ChE), albumin (ALB), iron, ferritin, and 2'-5' oligoadenylate synthetase (2-5 AS) were measured according to standard procedures. The peripheral blood leukocyte count (WBC), hemoglobin (Hgb), platelet count (PLT), and prothrombin time (PT) were also measured according to standard procedures.

5)Measurement of serum cytokines

Before starting IFN therapy, the serum levels of interleukin (IL)-10, IL-6, IL-18, TNF-alpha, and IFN-gamma were measured by ELISA with an IL-10 assay kit (Cytoscreen・US™, BioSource Int. Co., Ltd., Camarillo, CA, U.S.A.), IL-6 assay kit (QuantiGlo, R&D Co., Ltd., MN, U.S.A.), IL-18 assay kit (Hayashibara, Co., Inc. Okayama, Japan), TNF-alpha assay kit (QuantiGlo, R&D Co., Ltd., MN, U.S.A.), and human IFN-gamma assay kit (BMS228, Brender MedSystems Co., Ltd., Vienna Austria), respectively. We also calculated the serum IL-18/IL-10 ratio as the pro-inflammatory/anti-inflammatory ratio.

6)Statistical Analysis

Results are presented as the median value and the 10^{th}-90^{th} percentile. Baseline characteristics were compared among the three treatment groups by Tukey's test for continuous variables and chi-square test for categorical variables

Serum levels of AST, ALT, LDH, ferritin, HCV-RNA, 2-5 AS, and several cytokines were not normally distributed and therefore were logarithmically transformed. All pretreatment parameters were analyzed with the use of univariate logistic regression analysis. Multivariate logistic regression analysis was performed to determine the prognostic variables with SVR.

All tests were two-tailed, and a P value less than 0.05 was considered to indicate statistical significance.

Results

1)SVR

SVR was achieved in 64 patients (M/F: 44/20) with a median age of 50 years and a median baseline serum HCV-RNA level of 110 KIU/ml, while 23 patients (M/F: 13/10) with a median age of 52 years and median baseline serum HCV-RNA level of 225 KIU/ml were classified as NR. The overall SVR rate was 73 %, while it was 69 %, 76 %, and 76 % for the IFN-alpha group, C-IFN group, and R+IFN group, respectively (Table 2).

Table 2 Univariate logistic regression analysis

	SVR(n=64)	NR (n=23)	*P* value
No. of patients (n=)	64	23	
Age (y.o.)	Median: 50 (range: 34 - 67)	Median: 52 (range: 43 - 65)	0.110
Sex (M/F)	44 / 20	13 / 10	0.432
Previous IFN therapy (No/Yes)	54 / 10	18 / 5	0.507
Type of IFN Therapy			0.765
IFN-alpha alone (5 –10 MU)	25 (69%)	11 (31%)	
IFN-Con alone (12 –18 MU)	23 (76%)	7 (24%)	
IFN-α2b (6 MU) plus ribavirin	16 (76%)	5 (24%)	
Duration of the therapy	Median: 24 (range: 24 - 24)	Median: 24 (range: 18 - 24)	0.434
Baseline serum HCV (KIU/ml)	Median: 110 (range:11 - 488)	Median: 225 (range: 56 - 850)	0.010
Pathological findings			
Activity (1 / 2 / 3 / none)	18 / 38 / 7 / 1	5 / 11 / 6 / 1	0.265
Fibrosis (1 / 2 / 3 / 4 / none)	29 / 14 / 14 / 6 / 1	4 / 6 / 7 / 5 / 1	0.013

Range represents the 10th to 90th percentile.

Table 3 Univariate logistic regression analysis (laboratory data)

	SVR(n=64)	NR(n=23)	*P* value
Baseline peripheral blood			
WBC (×1000/mm^3)	Median: 5.50 (range:3.89 – 7.84)	Median: 4.65 (range: 2.15 – 6.30)	0.003
RBC (×10^4/mm^3)	Median: 444 (range:392 – 511)	Median: 443 (range:336 – 495)	0.219
Hgb (g/dl)	Median: 14.4 (range:12.2 – 16.0)	Median: 14.3 (range:11.7 – 15.9)	0.538
PLT (×10000/mm^3)	Median: 16.5 (range:9.2 – 24.4)	Median: 13.7 (range:5.4 – 22.7)	0.053
PT (%)	Median: 90 (range:78 – 105)	Median: 95 (range:75 –108)	0.516
Baseline serum parameters			
AST (IU/L)	Median: 67 (range:29- 169)	Median: 63 (range:32 - 140)	0.818
ALT (IU/L)	Median: 80 (range:27 - 247)	Median: 87 (range:28 - 237)	0.989
LDH (IU/L)	Median: 357 (range:283 –496)	Median: 332 (range:261 – 484)	0.632
ChE (IU/L)	Median: 292 (range:168 – 394)	Median: 287 (range:83 – 373)	0.603
Iron (μg/dl)	Median:130 (range:70 - 200)	Median: 153 (range:66 - 207)	0.187
Ferritin (ng/dl)	Median:261 (range:37 – 531)	Median:148 (range:61 - 565)	0.914
ALB (ng/dl)	Median:4.2 (range:3.7 – 4.7)	Median:4.4 (range:3.7 – 4.6)	0.473
2-5 AS (pmol/dl)	Median: 129 (range:61 – 271)	Median: 142 (range:45 – 227)	0.986
Baseline cytokines			
IL-6 (pg/ml)	Median:0.90 (range:0.24 – 4.02)	Median: 0.85 (range:0.31 – 4.05)	0.932
IL-10 (pg/ml)	Median:4.32 (range:0.50 – 10.8)	Median: 1.92 (range:0.60 – 4.50)	0.077
IL-18 (pg/ml)	Median: 257 (range:20 - 515)	Median: 283 (range:20 - 1028)	0.976
TNF-α (pg/ml)	Median: 1.58 (range:0.90 – 2.41)	Median: 2.13 (range:0.60 – 3.50)	0.197
IFN-γ (pg/ml)	Median:1.56 (range:1.56 – 5.33)	Median: 1.56 (range:1.56 – 6.35)	0.890
IL-18 / IL-10	Median: 51.8 (range:5.2 – 309.6)	Median: 123.7 (range:8.0 – 952.6)	0.039

Range represents the 10th to 90th percentile.

Table 4 Multivariate logistic regression analysis

Parameter	Estimate	S.E.	Waldχ^2	*P* value
Intercept	7.7392	3.2733	5.59	0.018
Log HCV-RNA	-3.1308	1.1249	7.75	0.005
IFN-alpha v.s. R+IFN	-2.6270	0.8208	10.24	0.001
IFN-alpha v.s. C-IFN	-2.9652	1.1321	6.86	0.009
Log IL-18 / IL-10	-1.5323	0.7022	4.76	0.029
Peripheral blood WBC	0.0007	0.0003	5.02	0.025

For log odds of SVR/NR

2)Univariate analysis

According to univariate analysis, the serum log-10 transformed HCV-RNA (log HCV-RNA) load, liver fibrosis, peripheral blood leukocyte count, and log-10 transformed IL-18/IL-10 ratio (log IL-18/IL-10 ratio) were associated with the achievement of SVR (Table 2,3).

3)Multivariate analysis

The multivariate logistic regression analysis selected the log HCV-RNA, log IL-18/IL-10 ratio, peripheral blood leukocyte count, and IFN regimen as significant parameters (Table 4).

Discussion

The present study revealed that the SVR rate achieved with IFN-alpha alone was lower than that for C-IFN alone or R+IFN. This result is related to the treatment period. Between 1997 and 2001, IFN-alpha alone was used to treat CHC patients regardless of their serum HCV-RNA level. The SVR rate achieved with C-IFN alone or R+IFN was an identical 76 %, probably because the C-IFN alone group included more patients with a lower viral load than the R+IFN group. On the other hand, the response of the R+IFN group was quite good, considering that this group contained more patients with a higher viral load. The SVR rate of the R+IFN group was the same as that achieved by a combination of PEG-IFN-alpha and ribavirin, which has currently become the standard therapy. CHC patients are now stratified according to their HCV genotype, and patients with genotypes 1 and 4 receive treatment for 48 weeks, while those with genotypes 2 and 3 are treated for 24 weeks. This combined therapy demonstrates greater efficacy, and SVR can be expected in 43-46 % of patients with genotypes 1 or 4, and in 75-80 % of those with genotypes 2 or 3[4]. However, the factors predicting the response to PEG-IFN plus ribavirin do not always include the serum viral load[14], suggesting that the efficacy of PEG-IFN plus ribavirin may depend more on host factors than viral factors.

IFN-based therapy is believed to influence the host immune response, rather than simply acting as an antiviral agent[5]. Since the factors associated with a lower or higher response to this treatment have not yet been fully identified, we performed a retrospective analysis with a multiple logistic regression model to identify pretreatment viral and host parameters that were associated with SVR to IFN-based therapy. As a result, the log serum HCV-RNA load, IFN regimen, log serum IL-18/IL-10 ratio, and peripheral blood leukocyte count were found to be independently associated with SVR according to both univariate and multivariate analysis.

The ratio of pro-inflammatory cytokines (such as IL-18) to anti-inflammatory cytokines (such as IL-10) has recently been reported to be a useful predictor of acute coronary syndrome[15,16]. The activity of specific T cells and non-antigen-specific NK cells is controlled by the Th1 immune response, and IL-18 has been found to be a strong co-factor in relation to acute coronary syndrome. IL-10 is an anti-inflammatory cytokine that inhibits pro-atherogenic processes via several mechanisms[17]. However, the significance of the IL-18/IL-10 ratio has not been investigated in CHC patients. HCV infection has been suggested to induce the production of IL-10 by regulatory T cells, which in turn suppresses CD4-positive T cell proliferation and may contribute to the persistence of HCV infection[18,19]. On the other hand, the serum IL-18 level was reported to increase after IFN-based therapy and indution of IL-18 is suggested to be related to the efficacy of IFN-based regimens[9,10]. It has been reported that IL-10 produced by regulatory T cells enhances NK-cell proliferation, cytotoxicity, and the production of IFN-gamma when combined with IL-18[20].

In the present study, the baseline serum levels of IL-18 and IL-10 were not selected as prognostic indicators, but the baseline serum IL-18/IL-10 ratio was selected by both univariate and multivariate regression analysis, suggesting that the ratio of these cytokines is more important for predicting the efficacy of IFN-based therapy than the absolute levels. Our results suggest that the decrease of pro-inflammatory cytokines such as IL-18 and increase of anti-inflammatory cytokines such as IL-10 induced by chronic HCV, i.e., an anti-inflammatory cytokine dominant state, might influence the response to IFN-based therapy. It is possible that IL-10 enhances the immune response in CHC patients when combined with IL-18, which are induced by IFN-based regimens during the early phase of therapy.

Another predictor of SVR selected by multivariate analysis was the peripheral blood WBC counts. It has been reported that the peripheral blood WBC count is lower in CHC patients than in controls[21]. In addition, it was reported that a higher peripheral blood WBC count in CHC patients 2 weeks after starting IFN-based therapy is a predictor of SVR[22], although the basis for this finding is not evident.

This study has the limitation that patients were treated with three different IFN-based regimens. Thus, further investigation of the baseline immune status of CHC patients receiving the same IFN-based regimen is needed, but it is possible that IFN-based therapy is more effective for patients with a higher peripheral blood leukocyte count and high levels of anti-inflammatory cytokines before therapy.

In conclusion, this is the first report (to our knowledge) that

the serum IL-18/IL-10 ratio and peripheral blood WBC count are host factors that are independent predictors of SVR in CHC patients with HCV genotype 2, along with the serum HCV-RNA load and treatment regimen. These factors may be critical determinants of SVR in CHC patients receiving PEG-IFN combined with ribavirin.

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