RAPID COMMUNICATION

Predictive factors for interferon and ribavirin combination therapy in patients with chronic hepatitis C

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Abstract

AIM: To confirm the predictive factors for interferon (IFN)-α and ribavirin combination therapy for chronic hepatitis patients with hepatitis C virus (HCV) genotype 1b.

METHODS: HCV RNA from 50 patients infected with HCV genotype 1b was studied by cloning and sequencing of interferon sensitivity determining region (ISDR), PKR-eIF2α phosphorylation homology domain (PePHD). Patients were treated with IFN-α and ribavirin for 6 mo and grouped by effectiveness of the therapy. A variety of factors were analyzed.

RESULTS: Our data showed that age, HCV RNA titer, and ISDR type could be used as the predictive factors for combined IFN-α and ribavirin efficacy. Characteristically, mutations in PePHD appeared only when the combination therapy was effective. Other factors, such as sex and alanine aminotransferase (ALT) level, were not related to its efficacy. Adjusting for age and HCV RNA titer indicated that the ISDR type was the most potent predictive factor.

CONCLUSION: HCV RNA ISDR type is an important factor for predicting efficacy of IFN-α and ribavirin combination therapy in Korean patients.

INTRODUCTION

Hepatitis C virus (HCV) is an enveloped RNA virus with a positive single-stranded RNA genome about 9600 nucleotides in length. This RNA encodes a single polypeptide approximately 3000 amino acids in length. The polypeptide is post-translationally cleaved into structural and non-structural proteins[1-3]. HCV infection is a major cause for chronic liver disease worldwide[4]. Eighty percent or more of acutely infected patients develop chronic hepatitis, which progresses to liver cirrhosis in about 20% of cases and hepatocellular carcinoma in 5%. These complications arise even if the disease remains asymptomatic[5].

Alpha-interferon (IFN-α) treatment effectively reduces viral load, but complete eradication of the virus is achieved in less than 20% of patients treated with IFN-α alone and in 40%-47% of patients treated with combined IFN-α and ribavirin[6-8]. The treatment outcome largely depends on the sensitivity of HCV genotype and IFN-α[9,10]. About 10 years before, IFN-α and ribavirin combination therapy has begun to be used instead of IFN monotherapy. Ribavirin is an oral nucleoside analogue acting on a broad spectrum of DNA and RNA viruses. It has been proposed to have both direct antiviral and immunomodulatory effects[11], but the detailed mechanism remains unclear. Ribavirin used as monotherapy is known to have little or no activity against HCV.

The HCV genotype appears to be a major determinant for IFN efficacy, because patients infected with HCV genotypes 2 and 3 respond better to IFN monotherapy than patients with genotype 1[9]. The IFN-based therapy effectiveness is not satisfactory, especially in patients with HCV genotype 1b[12-14], the most common genotype in Korea[15,16], Japan[17], southern and eastern Europe[18,19]. IFN is also inconvenient to use, costly, and has a variety

of possible complications. Thus, many studies have been carried out to determine the predictive factors for the efficacy IFN therapy[12-19].

Response to interferon monotherapy is associated with several host and viral factors. HCV genotype 1b, low viral load, and rapid HCV RNA clearance from the serum have been identified as favorable predictors for a sustained response to IFN therapy[20-22]. Since Enomoto *et al*[23] reported that genetic variability in a 40 amino acid stretch (amino acids 2209-2248) and mutations in the NS5A region of HCV and in designated interferon sensitivity determining region (ISDR), has become a predictive factor for IFN therapy. Studies from Japan[23], Sweden[24] and Spain[25] have shown that ISDR is an effective predictor. However, studies from Western countries displayed that ISDR is not a good predictor[26-28]. Studies on the relationship between IFN-α and ribavirin combination therapy and ISDR have controversial results[29-32].

Taylor *et al*[31] reported that a HCV envelope protein (E2) contains a sequence similar to the phosphorylation site on eIF2-α for the interferon-inducible cellular protein kinase PKR. The PKR-eIF2-α phosphorylation homology domain (PePHD) on E2 may serve as a pseudosubstrate for PKR and inhibit its function, reducing the antiviral effect of interferon. Thus, the PePHD region might also be involved in IFN resistance of chronic hepatitis C to IFN therapy. However, the role of this region is also controversial so far[31].

In Korea, the HCV prevalence is about 1%-2%, but studies to analyze the predictive factors for combined IFN-α and ribavirin therapy have not been performed. To identify these factors, we investigated the relationship between combined IFN-α and ribavirin efficacy and a variety of factors such as ISDR sequence, PePHD sequence, age, ALT level, and HCV RNA titer in Korean patients with HCV genotype 1b.

MATERIALS AND METHODS

Patents and treatment

Serum was collected from HCV genotype 1b-infected patients admitted to Wonju Christian Hospital. Only HCV genotype 1b was used in this study because it is the most common HCV genotype in the Republic of Korea. Sera were screened by a third generation ELISA method with an anti-HCV antibody. The patients were treated with IFN-α and ribavirin for 6 mo. Three million units of IFN-α was injected every two days, and 9 mg of ribavirin was orally administrated during the same period. The patients who did not receive the treatment were excluded. After the 6-mo combination therapy, the patients were classified into complete response group and no-response group. In the complete response group, HCV RNA titer was less than 50 IU/mL and ALT levels were within the normal range. In the no-response group, HCV RNA titer was over 50 IU/mL even if the ALT levels were normal.

cDNA preparation

HCV RNA was extracted from sera as previously described[33]. After ethanol precipitation, each RNA pellet was dissolved in 10 μL of diethylpyrocarbonate (DEPC)-treated distilled water for cDNA preparation. cDNA

synthesis was performed as previously described[34] with certain modifications. For the synthesis of cDNA of HCV, an aliquot of RNA (10 μL) isolated from the sera of patients was mixed with 1 μL of random hexamer (1 μmol/L), 2 μL of reaction buffer (250 mmol/L Tris-HCl pH 8.3, 250 mmol/L potassium chloride, 50 mmol/L magnesium chloride, 50 mmol/L dithiothreitol and 2.5 mmol/L spermidine) and 5.5 μL of DEPC-treated water was added. After the contents were heat-treated for 5 min at 65℃, 20 units (0.5 μL) of RNase inhibitor and 10 units (1 μL) of AMV reverse transcriptase were added. The mixture was incubated at 37℃ for 30 min, followed by at 99℃ for 1 min to inactivate the enzyme. PCR was performed as described previously[16]. The ISDR and PePHD primer sequences are listed in Table 1. PCR products were subjected to agarose gel electrophoresis in Tris-acetate-EDTA buffer and visualized with ethidium bromide staining under an ultraviolet transilluminator.

ISDR and PePHD sequencing

RT-PCR amplified products, including the ISDR and/or PePHD regions, were purified from agarose gel and glass milk (Gene Clean II kit, Bio 101, USA), and then subcloned by inserting the cDNA into a pGEM-T TA-cloning vector (Promega). The clones from each of the individual patient's plates were randomly selected and plasmid prepared from each clone was used as a template for DNA sequencing which was performed as previously described[35].

HCV RNA quantitation

In order to determine the HCV RNA titer, a quantitative and competitive polymerase chain reaction (QCPCR) assay was carried out as previously described[36]. As a first step, cDNA encoding the 5'-untranslated region of HCV was subcloned into a pGEM vector (pGEM5'UTR). Using PCR, the internal control plasmid, pGEM5'UTRDel, was constructed by deletion of nucleotides between the 87 and 165 nucleotides in the 5'-UTR of the HCV genome. The internal control RNA was synthesized *in vitro* by T7 RNA polymerase from a linearized template derived from the pGEM5'UTRDel plasmid. The amount of RNA synthesized *in vitro* was determined by measurement of the absorbance at 260 nm. A known copy number of the RNA was included as an internal control in order to quantify the viral RNA. The data were analyzed by Quantity One® 1-D analysis software (Bio-Rad).

Statistical analysis

Comparisons between groups were made by the Student's *t*-test. The *P* values were determined between the two groups with regard to age, ALT, amino acid mutations in PePHD, and HCV RNA titer. $P < 0.05$ was considered statistically significant. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for ISDR to test its predictive value for combination therapy by logistic regression analysis.

RESULTS

Patient characteristics

We collected serum from 50 HCV genotype 1b-infected

Table 1 Primer sequences used to amplify ISDR and PePHD

Region	Primer direction	Sequence (5' to 3')	Nucleotide No
ISDR	Outer sense	TGGATGGAGTGCGGTTGCACAGGTA	6703-6727
	Outer antisense	TGTAAAACGACGGCCAG	7296-7320
	Inner sense	TCTTTCTCCGTGGAGGTGGTATTGC	6722-6741
	Inner antisense	CAGGAAACAGCTATGACC	7275-7294
PePHD	Outer sense	TGACTACCCATACAGGCTCT	2180-2199
	Outer antisense	AAGGAAGGAGAGATTGCCAT	2725-2744
	Inner sense	AAGGTTAGGATGTATGTGGG	2238-2257
	Inner antisense	ATTGAGGACCACCGAGTTCT	2689-2708

ISDR: Interferon sensitivity determining region; PePHD: PKR-eIF2α phosphorylation homology domain; PKR: RNA-activated protein kinase.

Table 2 Characteristics of the complete response group patients before IFN-α and ribavirin combination therapy

	Age (yr)	Sex	ALT	Transfusion history[1]	HCV RNA titer[2]	ISDR Type[3]	No. of Amino Acid Mutations of PePHD
A-1	50	M	178	×	3.92	1	0
A-2	49	F	135	○	3.27	1	1
A-3	40	M	106	×	4.16	1	1
A-4	45	M	218	○	5.48	1	0
A-5	51	F	92	×	4.04	1	0
A-6	41	M	143	×	3.75	2	0
A-7	56	F	167	×	4.24	2	0
A-8	58	M	86	×	5.26	2	0
A-9	49	M	99	○	5.12	2	1
A-10	40	F	129	○	4.41	2	0
A-11	54	F	201	×	3.73	2	1
A-12	53	M	179	×	4.21	2	1
A-13	52	M	234	×	4.25	2	0
A-14	47	M	311	×	5.11	2	0
A-15	49	M	86	×	6.12	3	1
A-16	53	F	220	×	5.91	3	0
A-17	47	F	194	×	4.82	3	0
A-18	49	M	246	×	5.44	3	1
A-19	51	M	441	○	5.22	3	2
A-20	47	F	305	○	4.85	3	0
A-21	55	M	119	×	3.73	3	0

ISDR: Interferon sensitivity determining region; PePHD: PKR-eIF2α phosphorylation homology domain. [1]○ indicates history of transfusion and × indicates no history of transfusion. [2]The unit of HCV RNA titer before treatment (log copies/mL). [3]ISDR type: 1 (wild type, no amino acid substitution), 2 (intermediate type, 1-3 amino acid substitutions), 3 (mutant type, ⩾ 4 amino acid substitutions).

Table 3 Characteristics of the no-response group patients before IFN-α and ribavirin combination therapy

	Age (yr)	Sex	ALT	Transfusion history[1]	HCV RNA titer[2]	ISDR type[3]	No. of Amino Acid Mutations of PePHD
B-1	53	M	185	×	5.25	1	0
B-2	48	M	320	×	6.11	1	0
B-3	44	F	125	○	4.88	1	0
B-4	49	F	175	×	5.72	1	0
B-5	54	M	151	○	6.21	1	0
B-6	58	F	190	×	5.14	1	0
B-7	62	F	212	×	6.35	1	0
B-8	64	M	252	○	7.11	1	0
B-9	45	M	145	×	6.82	1	0
B-10	49	M	138	×	4.95	1	0
B-11	42	F	120	○	5.54	1	0
B-12	55	M	95	×	6.25	1	0
B-13	53	M	142	×	6.73	1	0
B-14	55	F	185	×	6.76	1	0
B-15	57	M	258	×	4.85	1	0
B-16	53	F	175	○	6.33	2	0
B-17	49	M	241	×	5.81	2	0
B-18	44	M	183	×	6.32	2	0
B-19	59	F	167	×	5.89	2	0
B-20	54	M	171	○	6.14	2	0
B-21	52	F	217	×	6.23	2	0
B-22	55	M	222	×	5.88	2	0
B-23	63	F	235	×	6.32	2	0
B-24	47	F	161	×	6.47	2	0
B-25	51	M	96	○	5.31	2	0
B-26	54	F	80	×	5.85	2	0
B-27	55	F	192	○	6.42	3	0
B-28	58	M	234	×	5.93	3	0
B-29	48	M	341	×	4.98	3	0

ALT: Alanine aminotransferase; ISDR: Interferon sensitivity determining region. [1]○ indicates history of transfusion and × indicates no history of transfusion. [2]The unit of HCV RNA titer before treatment (log copies/mL). [3]ISDR type: 1 (wild type, no amino acid substitution), 2 (intermediate type, 1-3 amino acid substitutions), 3 (mutant type, ⩾ 4 amino acid substitutions).

patients. Among them, 21 patients completely responded to the 6-mo combination therapy, while 29 patients showed no response. Group A was designated as the complete response group and group B as the no-response group. The characteristics of each patient prior to combination therapy are shown in Tables 2 and 3.

HCV RNA quantitation

As shown in Tables 2 and 3, the HCV RNA titer had a wide distribution. In the complete response group the HCV RNA titer was between $10^{3.27}$-$10^{6.12}$ copies per mL and $10^{4.85}$-$10^{7.11}$ copies per mL, respectively, in the no-response group. The average RNA titer of the response and no-response groups was 4.62 ± 0.80 and 5.59 ± 0.61, respectively. These values were statistically significant ($P < 0.05$, Table 4).

ISDR and PePHD amino acid sequences

The ISDR and PePHD amino acid sequences and the HCV genotype 1b prototype sequence (HCV-J) are shown in Figure 1. The complete response group had 1-10 amino acid substitutions while the no-response group had 1-8 amino acid substitutions in the ISDR (Figure 1A and B). The PePHD region had 1-2 amino acid substitutions in several cases of complete response group and no amino acid substitutions in the no-response group (Figure 2).

A

2209							2248
	PSLKA	TCTTH	HDSPD	ADLIE	ANLLW	RQEMG	GNITR	VESEN

	PSLKA	TCTTH	HDSPD	ADLIE	ANLLW	RQEMG	GNITR	VESEN
1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
4	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
5	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
6	- - - R -	- - - - -	- - N - -	P - - - -	- - - - -	- - - - -	- - - - -	- - - - -
7	- - - - -	- - - - R	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
8	- - - - -	- - - - -	- - - - -	V - - - -	- - - - -	- - - - -	- - - - -	- - - - -
9	- - - - -	- - - - -	- - - - -	P - - - -	- - - - -	- - - - -	- - - - -	- - - - -
10	- - - - -	- - - - R	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
11	- - - - -	- - - - C	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
12	- - - - -	- - - - R	- - - - -	V - - - -	- - - - -	- - - - -	- - - - -	- - - - -
13	L - - - -	- - - - -	- - - - -	I - - - D	- - - - -	- - - - -	- - - - -	- - - - -
14	- - - - -	- - - - -	- - - - -	L - - - -	- - - - -	- - - - -	- - - - -	- - - - -
15	V - - - -	A Y I - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
16	- - S - T	- Y I - -	R G - - -	- - - - -	- - - - -	- - - - -	- D - - -	- - - - -
17	L - - - -	A - - - N	- - - - -	V - - - -	- - - - -	- - - - -	- S - - -	- - - - -
18	V - - - -	A - - - -	- - - - -	- - - - -	- - - - -	- - - K -	- T - - -	- - - - K
19	L - - - -	- - R R -	- - - - -	- - D - -	- - - - -	W - - K -	- - - - -	- - - - -
20	L - - - -	- - - R -	N - - V -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
21	O - S - T	- Y I - Q	Y - G - -	V - - - -	- - - - -	- - - - -	- D - - -	- - - - -

B

2209							2248
	PSLKA	TCTTH	HDSPD	ADLIE	ANLLW	RQEMG	GNITR	VESEN

	PSLKA	TCTTH	HDSPD	ADLIE	ANLLW	RQEMG	GNITR	VESEN
1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
4	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
5	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
6	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
7	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
8	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
9	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
10	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
11	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
12	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
13	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
14	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
15	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
16	- - - - -	- - - - R	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
17	- - - - -	- - - - R	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
18	- - - - -	- - - - R	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
19	- - - - -	- - - - C	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
20	- - M - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
21	- - - - -	- - - - C	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
22	- - - - -	- - - - R	- V - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
23	- - - R -	- - - - R	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
24	L - - - -	- - - P -	- - - - -	- - - - -	- - - - -	W - - - -	- - - - -	- - - - -
25	- - - - -	- - - - R	- V - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
26	- - - - -	- - - - -	- - - - -	P - - - -	- - - - -	- - - - -	- - - - -	- - - - -
27	L - - - -	A - - A R	- G F I -	- - - - -	- - - - -	- - - - -	- E - - -	- - - - -
28	- - - - -	- G - - -	- - - - -	- - - - D	- - - - -	P - - - -	- D - - -	- - - - -
29	L - - - -	- - R R -	- - - - -	V - - - -	- - - - -	- - - - -	- - - - -	- - - - -

Figure 1 ISDR sequences (2209-2248) of HCV in complete response group (**A**) and in no-response group (**B**). Dashes indicate the amino acid residues identical to the sequences on the top in each panel.

Response to combination therapy in different groups

As shown in Table 4, patients were younger in the complete response group than in the no-response group. The HCV RNA titer was also significantly different. No differences were found in gender or ALT levels. We classified ISDR sequences into three groups based on the number of amino acid mutations, as previously described[23]. These three groups were analyzed by an odds ratio to define the responsiveness to combination therapy. The ISDR group responses to combination therapy are shown in Table 5.

Intermediate (one to three amino acid changes) and mutant (four or more amino acid changes) ISDRs showed an increased responsiveness to the combination therapy. The odds ratio was 2.46 and 7.00, respectively, assuming the wild type had 1.00. The age, PePHD mutations, and HCV RNA titer were significantly different between the two groups (Table 4). Considering these factors, ISDR type might be a better predictive factor for combination therapy responsiveness. After adjusting for age and HCV RNA titer, the odds ratio for intermediate and mutant ISDRs was 3.57

	Complete response group			No-response group		
	RS	ELSPL	LLSTT	RS	ELSPL	LLSTT
1	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
2	- A	- - - - -	- - - - -	- -	- - - - -	- - - - -
3	- Q	- - - - -	- - - - -	- -	- - - - -	- - - - -
4	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
5	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
6	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
7	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
8	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
9	- A	- - - - -	- - - - -	- -	- - - - -	- - - - -
10	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
11	- E	- - - - -	- - - - -	- -	- - - - -	- - - - -
12	- Q	- - - - -	- - - - -	- -	- - - - -	- - - - -
13	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
14	- -	- - - -	- - - - -	- -	- - - - -	- - - - -
15	- -	A - - - -	- - - - -	- -	- - - - -	- - - - -
16	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
17	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
18	- -	Q - - - -	- - - - -	- -	- - - - -	- - - - -
19	- A	Q - - - -	- - - - -	- -	- - - - -	- - - - -
20	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
21	- -	- - - - -	- - - - -	- -	- - - - -	- - - - -
22				- -	- - - - -	- - - - -
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Figure 2 PePHD sequences (659-670) of HCV in complete response group (n = 21) and no-response group (n = 29). Dashes indicate the amino acid residues identical to the sequences on the top in each panel.

Table 4 Statistical analysis of age, ALT and HCV RNA titer before treatment

Group	Male:Female	Age (yr)	ALT	HCV RNA titer before treatment
A	13:08	49.3 ± 5.0^a	185.2 ± 89.1	4.62 ± 0.80^b
B	16:13	52.8 ± 5.7^a	186.5 ± 61.7	5.95 ± 0.61^b

ALT: Alanine aminotransferase. The data of age, ALT and HCV RNA titer before treatment are shown in mean ± SD. A: Complete response group. B: No-response group. [a]P = 0.032 between A and B. [b]P = 0.001 between A and B.

Table 5 Logistic regression analysis of ISDR

ISDR type[1]	Crude OR	95% CI	Adjusted OR[2]	95% CI[2]
Wild	1.00		1.00	
Intermediate	2.46	0.64-9.39	3.57	0.66-19.36
Mutant	7.00	1.29-37.91	9.67	1.16-80.65

[1]ISDR wild type had no mutation. Intermediate type had 1-3 mutations and mutant type had more than 4 mutations. [2]After adjustment for age and HCV RNA titer before treatment. OR: Odds ratio; 95% CI: 95% confidence interval.

and 9.67, respectively. According to these results, patients with mutant ISDR strains would likely respond better to combination therapy than those with wild type ISDR strains.

DISCUSSION

Identifying host and viral factors can predict the response of HCV-infected patients to IFN-α and ribavirin combination therapy. Studies showed that factors such as the HCV genotype 1b and viral load are associated with resistance of HCV-infected patients to INF therapy[37,38]. It was reported that resistance of HCV genotype 1b-infected patients to INF therapy is influenced by a region of the NS5A viral phenotype[23,39]. Mutations in this region, known as ISDR, are beneficial for patients receiving IFN therapy, whereas the wild type virus is resistant to IFN treatment[23,39]. Other studies fail to confirm the association between ISDR genotypes and IFN responsiveness[26,27,40-44], thus it remains a controversial issue[45,46].

Combined IFN and ribavirin therapy has replaced IFN monotherapy for HCV-infected patients about 10 years before. In the present study, to identify the predictive factors for effective combination therapy, we investigated the relationship between the response to combination therapy and a variety of factors. Only patients with HCV genotype 1b were studied because this genotype is known to be more resistant to interferon treatment than the other genotypes and is the most prevalent genotype in Korea[15,16].

In this study, age, PePHD mutations, HCV RNA titer, and ISDR subtype were found to be the predictive factors for combined IFN-α and ribavirin therapy for HCV genotype 1b infection. On the other hand, gender and ALT level were not associated with the combination therapy efficacy. These results are consistent with many previous studies, but contrary to others[26,27,31]. Such a difference indicates that these factors are not always accurate predictors for IFN response. This effect may be due to the pleiotropic nature of IFN activity, in addition to other cellular and viral

genes that also modulate the effectiveness of INF therapy for chronic hepatitis C. This is the first study to determine the factors that predict the effectiveness of combination therapy in Korean patients. Therefore, this study may reflect the Korean genetic characteristics.

HCV seems to have a defense strategy against the host cellular responses induced by IFN[47]. The E2 protein appears to play a major role as a potential immune response target, and may interfere with cellular effectors induced by IFN[48]. Information about the clinical implications of E2 containing PePHD, is still limited. Analysis of a small series of HCV genotype 1-infected patients showed that amino acid sequence variability in the PePHD region was similar in responders and no-responders, indicating that the PePHD region is very stable over time[49-51]. In our study, a sequence analysis of the PePHD region in 50 patients found mutations in eight cases, all in the complete response group, suggesting that mutations in the PePHD region are associated with the response to combination therapy. In other studies, a few cases showed some PePHD mutations in the no-response group, though more mutations appeared in the complete response group[31,52]. Therefore, further study is needed to determine why mutations only occur in the complete response group of HCV-infected patients in Korea.

Some studies showed that the association of ISDR mutation rate with treatment response, but the other studies did not[31,45]. One of the Korean studies reported that the effect of INF monotherapy is not associated with the ISDR mutation rate[53]. It is not sure, but the different result may be due to the treatment methods and the sample size.

In conclusion, response of HCV genotype 1b-infected patients to combination therapy is influenced, at least in part, by HCV RNA titer, age, PePHD mutations, and ISDR subtype, but not by gender and ALT level. After adjusting for age and HCV RNA titer, ISDR subtype may be the most potent predictive factor for combination therapy efficacy in Korean chronic hepatitis patients with HCV genotype 1b.

COMMENTS

Background

The effectiveness of IFN-α and ribavirin combination therapy in Hepatitis C virus (HCV) infected patients is not satisfactory, especially in patients with HCV genotype 1b. IFN is also inconvenient to use, costly, and has a variety of possible complications. Therefore, it is necessary to determine the predictive factors for IFN therapy.

Research frontiers

It is very important to know the pathogenesis of HCV-infected patients to block the progression of hepatocellular carcinoma.

Innovations and breakthroughs

This is the first study to determine the factors that predict the effectiveness of IFN-α and ribavirin combination therapy in Korean patients. In this study we used a large number of samples to determine the ISDR subtype, HCV RNA titer, age, and PePHD mutations.

Applications

The predictive factors for IFN-α and ribavirin combination therapy in patients with HCV genotype 1b can be used to select its candidates.

Peer review

This article is of theoretical and practical importance. The results show that HCV RNA ISDR type may be an important factor for predicting the efficacy of IFN-α and ribavirin combination therapy in Korean patients.

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