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Polymorphisms in the interferon-γ gene at position +874 in patients with chronic hepatitis C treated with high-dose interferon-α and ribavirin

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Abstract

To investigate the influence of the T-to-A polymorphic sequence at position +874 in the interferon (IFN)-γ gene (+874 IFN-γ) on the response to combination therapy with high-dose interferon and ribavirin, the single nucleotide polymorphisms were determined by using a polymerase chain reaction sequence-specific primers approach in 150 histologically proved chronic hepatitis C (CHC) patients. The distribution of genotypes for +874 IFN-γ were T/T: 6 (4.0%), T/A: 31 (20.7%) and A/A: 113 (75.3%) and 24.7% (37/150) of patients were inherited T allele. After undergoing combination therapy with high-dose IFN-α and ribavirin, 70.7% (106/150) of patients achieved sustained viral response (SVR). Based on multivariate regression analyses, the independent factors predicting HCV SVR after combination therapy were HCV genotype non-1b ($P < 0.001$) and low pretreatment HCV RNA levels ($P = 0.041$) (odds ratios/95% C.I.: 10.150/4.023–25.609 and 0.581/0.345–0.979, respectively). No association between genotypes, A or T alleles of +874 IFN-γ and response to combination therapy with high-dose IFN-α and ribavirin.

In conclusion, we found that with high SVR rates after combination therapy with high-dose IFN-α and ribavirin, HCV genotypes and pretreatment serum HCV RNA levels, but not inheritance of the IFN-γ polymorphism at the position +847, were predictors for SVR.

Keywords: Combination therapy; Hepatitis C virus; IFN-α; IFN-γ; Polymorphism; Ribavirin

1. Introduction

Being the major etiologic agent in parenterally transmitted non-A non-B hepatitis, hepatitis C virus (HCV) infection has variable clinical course and frequently causes persistent infection leading to chronic liver disease and primary hepatocellular carcinoma (Alter et al., 1992). Interferon (IFN)-α has direct antiviral effects and a number of immunomodulatory activities that can enhance antiviral immune responses (Peters, 1996). Treatment with IFN-α was the first approved therapy but a sustained virological response could be achieved in only 20–35% of patients with IFN-α monotherapy (Lauer and Walker, 2001; Yu et al., 2000). Combination therapy with IFN-α and ribavirin achieves better sustained viral response (31–43% for 24 or 48 weeks therapy) than IFN-α monotherapy, and has become the standard of therapy for CHC patients (McHutchison et al., 1998; Poynard et al., 1998). Some virologic factors have been indicated as predictors of the response to IFN-α therapy (Lauer and Walker, 2001; Martinot-Peignoux et al., 1995; Poynard et al., 1998). Response to therapy may also be determined by the host immune response (Koziel, 1999).

The polarization of immune system toward either a predominantly cellular (TH1) or humoral (TH2) response

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depends largely upon the cytokines present at the site of immune activation. The single-nucleotide polymorphisms (SNPs) in regulatory regions of cytokine genes have been demonstrated to affect the levels of expression of some cytokines. Hence, genetic differences affecting cytokine expression could influence the strength and duration of the immune response and even predispose to immunologic disorders and allograft rejection (Awad et al., 1998; Fishman et al., 1998). The genetic polymorphism has been reported associated with chronic hepatitis B (Hohler et al., 1998). In HCV infection, the production of inappropriate levels of some cytokines is reported to contribute to viral persistence and to affect response to therapy (Larrea et al., 1996; Nelson et al., 1997; Tilg et al., 1992). The role of polymorphisms in cytokine genes in the pathogenesis of HCV infection also has shown possibly associated with susceptibility or response to therapy (Tambur et al., 2001; Vidigal et al., 2002; Yee et al., 2001).

IFN-γ, a representative TH1 cytokine plays an important role in defense against viruses and intracellular pathogens and in the induction of immune-mediated inflammatory responses. It has been reported that IFN-γ production is genetically controlled. The T to A polymorphism located at position +874 of the intron 1 of IFN-γ gene (+874 IFN-γ) has been considered directly influencing the level of IFN-γ production associated with the CA microsatellite marker (Pravica et al., 2000). Three possible genotypes are generated by analyzing the sequence mutation for +874 IFN-γ (either T or A), the A/A, T/A and T/T genotypes, which were thought to confer three different phenotypes: low, intermediate and high producers of IFN-γ, respectively (Perrey et al., 1998; Tambur et al., 2001). Polymorphism in IFN-γ gene has been reported to be associated with lung allograft fibrosis (Awad et al., 1999). Nevertheless, little is known about the effect of this SNP on the HCV response to therapy to date.

Taiwan is a hyperendemic country for hepatitis B virus (HBV) infection and several HCV hyperendemic townships have been discovered with the anti-HCV prevalence more than 15% in southern Taiwan (Wang et al., 1999, 2002). Favorable results have been reported among Taiwanese naïve CHC patients after IFN-α monotherapy or combination therapy with IFN-α and ribavirin (Dai et al., 2002, 2003; Lai et al., 1996). IFN-α therapy may modulate T-cell function and cytokine production, which may be one of the mechanisms in reducing viral burden (Cacciarelli et al., 1996). The nature of a TH1 lymphocyte response has been shown to be important in HCV infection. IFN-γ has been reported to be associated with control of HCV replication and disease progression (Lechmann et al., 1999; Cramp et al., 1999). The aims of this study were to determine allele frequencies in the +874 IFN-γ among Taiwanese naïve CHC patients and whether the polymorphism influences the response to combination therapy with high-dose IFN-α and ribavirin.

2. Materials and methods

2.1. Patients

One hundred and fifty naïve patients diagnosed as chronic hepatitis C patients (93 males and 57 females, mean age: 46.8 ± 11.3 years) at the Kaohsiung Medical University Hospital were enrolled in the study. All patients were positive for HCV antibodies and serum HCV RNA, negative for hepatitis B surface antigen (HBsAg) and received liver biopsies. Liver histology was assessed blindly by two pathologists and disease activity grade and fibrosis stage were quantitatively scored according to the histological activity index (HAI) (Knodell et al., 1981). The present study was approved by the ethics committee of Kaohsiung Medical University Hospital.

2.2. Laboratory tests

Second-generation HCV antibody (anti-HCV) was detected with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Abbott, North Chicago, IL, USA). Serum HBsAg was assayed using commercially available kits (General Biological HBsAg radio-immunoassary (RIA); General Biological Cooperation, Taiwan). Alanine aminotransferase (ALT) was measured on a multichannel autoanalyzer. Detection of serum HCV RNA was performed using a standardized automated qualitative reverse transcription polymerase chain reaction assay (COBAS AMPLICOR Hepatitis C Virus Test, version 2.0; Roche, Branchburg, NJ, USA). The detection limit was 50 IU/mL. HCV genotypes 1a, 1b, 2a, 2b and 3a were determined by amplification of the core region using genotype-specific primers (Okamoto et al., 1993). Pretreatment HCV RNA levels were determined by using the branched DNA assay (Quantiplex HCV RNA 3.0, Bayer, Emeryville, CA, USA) performed strictly in accordance with the manufacturer's instructions. The quantification limit was 615 IU of HCV RNA per mL.

2.3. Polymerase chain reaction sequence-specific primers (PCR-SSP) typing

The single nucleotide allelic variation of +874 INF-γ (either T or A) was analyzed by using a PCR-SSP approach: the homozygous T produces high levels of IFN-γ; the heterozygous T/A is an intermediate producer; and the homozygous A genotype represents the potential to generate only low amounts of IFN-γ (Pravica et al., 1999). PCR amplification of IFN-γ alleles and an internal control, the human β-globin gene was carried out according to manufacturer's recommendations (One Lambda Inc., Canoga Park, CA, USA). Briefly, after the addition of the appropriate primer pairs, salts, buffer and Taq polymerase, the samples were subjected to 30 cycles of PCR as follows: one cycle of 130 s at 96 °C, dropping to 63 °C for an additional 60 s; 9 cycles of 10 s at 96 °C,

60 s at 63 °C; and the final 20 cycles which included a three-temperature ramp-annealing for 10 s at 96 °C, hybridization for 50 s at 59 °C, and an extension step of 30 s at 72 °C. PCR products were then loaded onto an agarose gel and photographed with an ultraviolet transilluminator.

2.4. Combination therapy with high-dose IFN and ribavirin

After all 150 patients had provided informed consent, they were treated with combination therapy with high-dose recombinant IFN-α administered intramuscularly, 6 MU thrice weekly, and oral ribavirin 1000–1200 mg administered daily for 24 weeks. The presence of HCV RNA in the serum was assessed every 3 months to determine the responses to combination therapy. SVR was defined as clearance of serum HCV RNA at the end of the therapy as well as at 24 weeks after the cessation of therapy. All other patients were classified as non-responders (NRs).

2.5. Statistical analyses

Frequency was compared between groups using the chi-square test with Yate's correction or Fisher's exact test. Group means were compared using the Student *t*-test. Serum HCV RNA levels was expressed as the mean ± S.D. after logarithmic transformation of original values. Stepwise logistic regression was used to analyze which variables had a better predictive value for SVR. The procedures were performed using the SPSS 12.0 statistical package (SPSS Inc., Chicago, IL, USA). All statistical analyses are based on two-side hypothesis tests with a significance level of $P < 0.05$.

3. Results

Demographic, clinical and virological features of the 150 CHC patients are shown in Table 1. Sixty-nine patients (46%) were deduced to have HCV genotype 1b and 15 (10%) patients were diagnosed as liver cirrhosis. The distribution of genotypes for +874 IFN-γ were T/T: 6 (4.0%), T/A: 31 (20.7%) and A/A: 113 (75.3%). The frequency of the T allele was 14.3% and 24.7% (37/150) of patients inherited T allele.

One hundred and six (70.7%) of the 150 patients achieved SVR after undergoing combination therapy with high-dose IFN-α and ribavirin. Comparisons of clinical characteristics between patients with and without SVR are shown in Table 2. In univariate analyses, SVR was significantly related to HCV genotype non-1b ($P < 0.001$) and lower pretreatment levels of HCV RNA ($P = 0.009$). Patients with SVR had higher mean ALT levels than patients without SVR, the difference, however, was not statistical significance (111.4 ± 86.1 versus 65.8 ± 201.6 IU/L, $P = 0.086$). Based on multivariate regression analyses, the independent factors predicting HCV SVR after combination therapy were HCV genotype non-1b ($P < 0.001$) and low pretreatment HCV RNA levels ($P = 0.041$) (odds ratios/95% C.I.: 10.150/4.023–25.609 and 0.581/0.345–0.979, respectively). No association between genotypes, A or T alleles at position +874 in IFN-γ and response to combination therapy with high-dose IFN-α and ribavirin.

Table 1
Characteristics of 150 chronic hepatitis C patients receiving combination therapy with high-dose interferon-α and ribavirin for 24 weeks

Sex (male, %)	93 (62)
Age (year)[a]	46.8 ± 11.3
Pretreatment serum levels of ALT (IU/L)[a]	149.8 ± 177.1
Pretreatment HCV RNA level (log IU/mL)[a]	5.52 ± 0.89
HCV genotype (%)	
1b	69 (46.0%)
2a	43 (28.7%)
2b	17 (11.3%)
Mixed	5 (3.3%)
Unclassified	16 (10.7%)
Histological activity index	
Necroinflammatory activity[a]	4.06 ± 2.62
Fibrosis[a]	1.32 ± 1.34
Liver cirrhosis (%)	15 (10)

HCV: hepatitis C virus; ALT: alanine aminotransferase.
[a] Presented as mean ± standard deviation.

Table 2
Factors associated with response to combination therapy with high-dose interferon-α and ribavirin in 150 chronic hepatitis C patients

Factors	NR (*n*, %) (*N* = 44)	SVR (*n*, %) (*N* = 106)	*P*
Sex (male)	29 (65.9)	64 (60.4)	0.525
Age (year)[a]	47.9 ± 9.9	46.4 ± 11.9	0.477
HCV genotype 1b	36 (81.8)	33 (31.1)	**<0.001**
Pretreatment serum HCV RNA levels (log IU/mL)[a]	5.82 ± 0.63	5.40 ± 0.96	**0.009**
Pretreatment serum ALT levels (IU/L)[a]	111.4 ± 86.1	165.8 ± 201.6	0.087
Histological activity index			
Total score[a]	3.91 ± 2.25	4.12 ± 2.76	0.651
Fibrosis score[a]	1.59 ± 1.39	1.21 ± 1.31	0.110
Liver cirrhosis	6 (13.6)	9 (8.5)	0.375
+874 IFN-γ genotype			0.646
A/A	35 (79.5)	78 (73.6)	
T/A	7 (15.9)	24 (22.6)	
T/T	2 (4.5)	4 (3.8)	
+874 IFN-γ A allele			1.0
Positive	42 (29.2)	102 (70.8)	
Negative	2 (33.3)	4 (66.7)	
+874 IFN-γ T allele			0.441
Positive	9 (24.3)	28 (75.7)	
Negative	35 (26.4)	78 (76.3)	

HCV: hepatitis C virus; NR: non-responder; SVR: sustained viral responder; ALT: alanine aminotransferase; +874 IFN-γ: interferon-γ gene at position +874.
[a] Presented as mean ± standard deviation.

4. Discussion

Among naïve Taiwanese CHC patients, we have reported the SVR rates using high-dose (6 MU) IFN-α monotherapy achieved 36–41% (Dai et al., 2002, 2003). With the report from Lai et al. showing 43% of Taiwanese naïve CHC patients achieving SVR after combination therapy of IFN-α 3 MU and ribavirin for 24 weeks (Lai et al., 1996), we further retreated CHC patients failing to respond to previous IFN-α monotherapy using combination therapy with high-dose (6 MU) IFN-α and ribavirin therapy and obtained a 53.3% SVR rate (Chuang et al., 2004). In the present study, the high SVR rate for naïve Taiwanese CHC patients achieved 70.7%, which was similar to our previous reports (Dai et al., 2004). The favorable responses to IFN-α, either monotherapy or combined with ribavirin may encourage physicians to treat CHC patients more aggressively in Taiwan.

In HCV infection, the production of inappropriate levels of some cytokines such as tumor necrosis factor-alpha and IL-10 is reported to contribute to viral persistence and to affect response to therapy (Larrea et al., 1996; Nelson et al., 1997; Tilg et al., 1992). Besides, the role of polymorphisms in genes of transforming growth factor-beta1, IL-6 and IL-10 in the pathogenesis of HCV infection also has shown possibly associated with susceptibility or response to therapy (Tambur et al., 2001; Vidigal et al., 2002; Yee et al., 2001). In our previous study, we have identified the polymorphisms of tumor necrosis factor alpha promoter at position −308 is an independent predictor for response to combination therapy, especially in genotype 1b-infected patients (unpublished data). Since the IFN-γ production is influenced by the polymorphism located at the +874 position, whether the alleles of +874 IFN-γ is associated with responses to therapy needs to be clarified. This study determined allele frequencies in the +874 IFN-γ among Taiwanese CHC patients and investigated the influences of +874 IFN-γ gene polymorphisms on the HCV response to combination therapy with high-dose IFN and ribavirin. In surveying the clinical factors associated with SVR to combination therapy with high-dose IFN-α and ribavirin, we confirm the HCV genotype non-1b and lower pretreatment serum HCV RNA levels, in agreement with previous reports (Martinot-Peignoux et al., 1995; Poynard et al., 1998), are predictors for the SVR. In the present study, no association between +874 IFN-γ gene polymorphisms and HCV response was found. To evaluate HCV genotype and viral load before treatment is critical for choosing an adequate regimen and the determination of +874 IFN-γ polymorphisms, which has no importance in predicting of response in CHC patients.

In conclusion, we found that with high SVR rates after combination therapy with high-dose IFN-α and ribavirin, HCV genotypes and pretreatment serum HCV RNA levels, but not inheritance of the IFN-γ polymorphism at the position +847, were predictors for SVR.

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