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MAJOR ARTICLE

Interferon and Ribavirin Therapy for Chronic Hepatitis C Virus Genotype 6: A Comparison with Genotype 1

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Because there is a lack of data on the treatment outcome of patients who carry hepatitis C virus (HCV) genotype 6, we conducted a prospective study, to compare the effect of interferon and ribavirin therapy in HCV genotypes 1 and 6, of patients with seropositive anti-HCV, persistently elevated alanine transaminase levels, and detectable HCV RNA. Patients were treated with subcutaneous recombinant interferon α-2b and ribavirin for 12 months. Of 40 patients, 16 had genotype 6, and 24 had genotype 1. An end-of-treatment response was detected in 12 (75%) patients with genotype 6 and in 10 (41.6%) patients with genotype 1 ($P = .05$). A sustained virological response (SVR) was present in 10 (62.5%) patients with genotype 6 and in 7 (29.2%) patients with genotype 1 ($P = .04$). Genotype 6 has a better response than genotype 1 and is associated with a higher SVR.

Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide. HCV can induce chronic infection in ~85% of infected individuals [1]. Studies of HCV isolates collected worldwide have documented significant genetic variations [2]. On the basis of their genetic heterogeneity, it is now generally accepted that HCV has evolved into 6 major genotypes, designated, in order of discovery, as 1–6.

In Western Europe and the United States, the predominant genotypes are 1a, 1b, 2b, and 3a, with some variation in frequency [3, 4]. In Japan and Taiwan, types 1b, 2a, and 2b are seen most frequently [4]. Elsewhere in Asia, genotype 3 is the most common, whereas genotype 4 is found most frequently in the Middle East and Africa [3, 4]. In Hong Kong, types 1a, 1b, 2b, 3, and 6 have been reported to be the most common genotypes [3, 4]. Of these 6 major genotypes, type 6a shows one of the most confined geographical locations, having been found only in Hong Kong, Macau, and Vietnam or in emigrants from these countries [5]. Genotype 6 has also been reported in Thailand [5].

As far as treatment is concerned, a number of studies have demonstrated that pretreatment virus load and genotype are independent predictors for the response to treatment with interferon (IFN). The presence of HCV genotype 2 or 3 has been associated with a greater response to IFN, compared with genotype 1 [6, 7].

However, there has been a lack of data on the effect of IFN and ribavirin in patients who have chronic hepatitis C disease with genotype 6, mainly because they are predominantly found in Southeast Asia. We, therefore, conducted a study to compare the effect of IFN and ribavirin therapy in HCV genotypes 1 and 6.

PATIENTS AND METHODS

From January 1999 to February 2001, Chinese patients with chronic hepatitis C disease were recruited into our

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The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Hong Kong, and written informed consent was obtained from each patient.

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prospective trial at the Department of Medicine, Queen Mary Hospital, the University of Hong Kong (Hong Kong, China). Patients recruited into the study fulfilled the following criteria: (1) seropositive for anti-HCV by the third-generation ELISA (Ortho Diagnostic Systems), (2) positive for HCV RNA by use of a branched DNA assay (Quantiplex HCV-RNA 2.0 assay; Chiron), (3) persistently elevated alanine transaminase (ALT) levels (normal range, 6–53 U/L) on at least 3 occasions for at least 6 months, and (4) features of chronic inflammation on pretreatment liver biopsy specimens. Patients with advanced liver cirrhosis, hemolytic disease, depression, autoimmune diseases, hepatitis B virus infection, human immunodeficiency virus (HIV) infection, previous IFN therapy, and pregnancy were excluded.

The HCV genotypes were determined using the VERSANT HCV Genotype Assay (Bayer), following the manufacturer's instructions. This line-probe assay contains specific probes, which allows the determination of HCV genotypes 1–6 and its major subtypes [8]. Patients with genotype 1 recruited into the present study had their samples retested by amplification of part of the HCV NS5B region and subsequent phylogenetic analysis that used the GENEBASE program (Applied Maths) to confirm that the samples were not genotypes 7, 8, or 9 mistyped as genotype 1, because this can affect the response of treatment [9]. For phylogenetic analysis, a list of reference sequences was selected from well-characterized full-genome sequences in the GenBank database, covering all known genotypes and the major subtypes.

Patients recruited into the present study were treated with subcutaneous recombinant IFN α-2b (Schering-Plough), 5 mU 3 times weekly, and oral ribavirin (Schering-Plough) 1000 or 1200 mg daily in 2 divided doses (1000 mg for those weighing <75 kg and 1200 mg for those weighing >75 kg) for 12 months.

A percutaneous liver biopsy was performed at 1 month before treatment and 6 months after treatment. The specimens were formalin fixed and reviewed by a pathologist blinded to the biochemical, genotype, and HCV RNA data, according to the method of Desmet et al. [10]. Improvement or progression in posttreatment liver biopsy samples was classified as a change in the score of $\geqslant 1$.

The patients were followed-up regularly every 4 weeks while receiving treatment and at 3 monthly intervals after treatment. During each follow-up, complete blood counts, liver biochemistry, thyroid function test, and HCV RNA were checked. The end-of-treatment response (ETR) was defined as undetectable HCV RNA at the end of treatment. A sustained virological response (SVR) was defined as undetectable HCV RNA 6 months after the completion of treatment.

All statistical analyses were performed using SPSS 10.0 for windows (SPSS). The Mann-Whitney U test was used for continuous variables with skewed distribution, a χ^2 test with Yates's correction and Fisher's exact test were used for categorical variables. All statistics were performed on the intent-to-treat population. Statistical significance was defined as $P < .05$.

RESULTS

During this period, there were 196 anti-HCV–positive patients with detectable HCV RNA being monitored in our clinic. Among these patients, the genotypes detected were 1a (8.1%), 1b (54.7%), 2a (2.6%), 2b (0.5%), 3a (1.5%), 6a (21.9%), and

Table 1. Baseline demographic data of patients with hepatitis C virus (HCV) genotypes 6 and 1.

Factors	Genotype 6 (n = 16)	Genotype 1 (n = 24)	P
Age, years (range)	44 (29–51)	50.5 (24–80)	.06
Sex, M:F	12:4	18:6	.26
Bilirubin, μmol/L	10 (5–30)	10 (4–36)	.87
ALP level, U/L	77 (60–111)	71 (48–106)	.11
AST level, U/L	83 (39–275)	57 (33–290)	.32
ALT level, U/L	120 (61–298)	90 (37–588)	.17
HCV RNA level, $\times 10^6$ Eq/mL	6.05 (1–28.6)	1.015 (0.3–44.75)	.22
Inflammatory activity before treatment, no. (%) of subjects			.72
0–1	13 (81.3)	18 (75)	
2–3	3 (18.8)	6 (25)	
Stage of fibrosis before treatment, no. (%) of subjects			.60
0–2	10 (62.5)	13 (54.2)	
3	6 (37.5)	11 (45.8)	

NOTE. ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase.

Table 2. Baseline demographic data of patients with hepatitis C virus (HCV) genotypes 6 and 1 who were not recruited into the study.

Factor	Genotype 6 (n = 13)	Genotype 1 (n = 20)
Age, years (range)	53 (30–64)	57 (28–84)
Sex, M:F	9:4	12:8
Bilirubin level, μmol/L	9 (6–31)	12 (5–40)
ALP level, U/L	82 (65–124)	77 (41–123)
AST level, U/L	81 (49–269)	64 (45–278)
ALT level, U/L	143 (59–311)	110 (46–511)
HCV RNA level, $\times 10^6$ Eq/mL	7.10 (1.2–26.9)	1.21 (0.6–38.50)

NOTE. No significant difference was detected in the baseline demographic data between patients who did and did not receive therapy with genotypes 1 or 6. ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase.

6b (5.1%). Mixed and indeterminate genotypes were detected in 5.6%. Of the 176 patients with genotypes 1 and 6, 56 (31.8%) had normal ALT levels, 32 (18.2%) had previously received IFN monotherapy, 6 (3.4%) had liver cirrhosis, 3 (1.7%) had undergone liver transplantation, 3 (1.7%) had a history of Graves disease, 2 (1.1%) had depression, and 1 (0.6%) had undergone renal transplantation. Of the 73 patients who fulfilled the entry criteria for our study, 40 (54.8%) were recruited—24 with genotype 1 and 16 with genotype 6. The pretreatment demographic data between patients with genotypes 1 and 6 are shown in table 1. There were no significant differences between the 2 groups (all P values were not significant [NS]). Even though genotype 1 had a higher proportion of patients with F3 fibrosis, compared with genotype 6, this difference was not significant (P, NS).

The baseline demographic data for patients with genotypes 6 and 1 who were not recruited into our study are shown in table 2. No significant difference was detected in the baseline demographic data between patients with genotype 1 who did and did not receive therapy (all P, NS). No significant difference was also detected in the baseline demographic data between patients with genotype 6 who did and did not receive therapy (all P, NS).

At 24 weeks of treatment, 11 patients (68.8%) with genotype 6 had undetectable HCV RNA, whereas, of those with genotype 1, 8 patients (33.3%) had undetectable HCV RNA. A significant difference could be detected when genotype 6 was compared with genotype 1 ($P = .02$; odds ratio [OR], 4.40; 95% confidence interval [CI], 1.13–17.07).

At the end of treatment, 12 patients (75%) with genotype 6 had an ETR, whereas 10 patients (41.6%) with genotype 1 had an ETR. There was a marginally significant difference in the ETR between genotypes 6 and 1 ($P = .05$; OR, 4.20; 95% CI, 1.04–16.90).

An SVR was present in 10 (62.5%) patients with genotype 6 and in 7 (29.2%) patients with genotype 1. A significant difference between the SVR in patients with genotypes 6 and 1 could be detected ($P = .04$; OR, 4.05; 95% CI, 1.06–15.48).

Posttreatment liver biopsy specimens showed improvement in inflammatory activity in 4 (25%) patients with genotype 6 and in 3 (12.5%) patients with genotype 1 ($P = .41$). There was an improvement in the stage of fibrosis in 4 (25%) patients with genotype 6 and in 2 (8.3%) patients with genotype 1 ($P = .20$). Progression in the histology samples was detected in only 1 (6.3%) patient with genotype 6, whereas 3 (12.5%) patients with genotype 1 had progression, as detected on liver biopsy samples ($P = .64$). One (4.2%) patient with genotype 1 developed cirrhosis, whereas no patients with genotype 6 had developed liver cirrhosis by the end of the study.

Five (12.5%) patients had a decrease in their hemoglobin levels by 2 g/dL, but they did not require a reduction in the dose of ribavirin. Only 2 (5%) patients had a decrease in hemoglobin levels to <10 g/dL and required a reduction in the dose of ribavirin. Three (7.5%) patients were withdrawn from treatment because of thyrotoxicosis (2 patients) and depression (1 patient).

DISCUSSION

In patients with chronic hepatitis C infection, combination therapy with IFN and ribavirin for 48 weeks has an SVR rate of 28%–31% in patients with genotype 1 and 64%–66% in patients with genotype 2 or 3 [6, 7]. In those 2 studies, it was also found that 64%–69% of patients with genotype 2 or 3 had a sustained response to combination therapy, even with 6 months of treatment, whereas genotype 4 has been shown to be associated with a poor SVR rate to combination therapy of only 20% [11].

On the basis of these studies, it has been deduced that the response of HCV to IFN and ribavirin is dependent on the genotype. So far, there has been no study on the response to combination therapy in patients with chronic HCV genotype 6. In Hong Kong, genotype 6 occurs in 27% of patients with chronic HCV, and genotype 1b can be found in 58.8% [12]. Genotype 6 was reported to be particularly prominent among patients with thalassaemia major and intravenous drug abusers [13]. Genotypes 1 and 6 were the 2 most common genotypes in our center, accounting for 62.8% and 27%, respectively, of our patient population. In view of this, there is a need to determine the response of combination IFN and ribavirin therapy in genotypes 6 and 1 in Chinese people.

Our study compared the response to treatment for patients with HCV genotypes 6 and 1. The dose of IFN used in the present study was higher than that used in the 2 major registration trials [6, 7] of combination therapy for chronic hepatitis

C disease, because there were data showing that a higher dose of IFN may be more effective in the treatment of patients with unfavorable viral characteristics [14]. As shown in table 1, there were no significant differences in the demographic data of patients with genotype 6 versus those with genotype 1 in the present study. In fact, the level of HCV viremia in patients with genotype 6 in our study tended to be higher than that of genotype 1, although this difference was not significant.

Our study showed that the ETR and SVR in genotype 6 were 75% and 62.5%, whereas, in genotype 1, they were only ~41.6% and 29.2%. This is similar to the response rate of genotype 1 from previous studies, even though the dose of IFN used in the present study was 60% higher [6, 7]. This shows that, unlike black Americans with genotype 1, who were less likely than white Americans to respond to IFN treatment [15], Chinese people with genotype 1 were not more refractory to treatment and that a higher dose of IFN does not lead to a better SVR in Chinese patients with genotype 1. Because the baseline characteristics between the 2 genotypes were not significantly different, we can safely conclude that it is the genotype that plays a more important role in determining response to therapy. To the best of our knowledge, the present study is the largest to have documented the response of genotype 6 to therapy. It is also the largest study to have documented the response of Chinese people with genotype 1 to therapy.

The study has a few limitations. First, there was a lack of patients with genotype 6 randomized to receive 6 months of therapy in order to determine the optimum duration of therapy for genotype 6. Second, the number of patients studied was small. Third, patients with genotype 1 were older than those with genotype 6, even though this difference was not significant.

In conclusion, HCV genotype 6 has a better response to IFN treatment than HCV genotype 1 and is associated with a significantly higher SVR. A higher dose of IFN does not seem to increase the SVR rate in Chinese patients with genotype 1. Future trials should be performed to determine whether 6 months of combination therapy is adequate for patients with genotype 6.

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