• BRIEF REPORTS •

Response of TT virus to IFN plus ribavirin treatment in patients with chronic hepatitis C

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Abstract

AIM: TT virus (TTV) is a newly described DNA virus related to postransfusion hepatitis that produces persistent viremia in the absence of clinical manifestations. PEG-IFN plus ribavirin have been useful in the treatment of chronic hepatitis C infection. This study investigated the responses of TT virus (TTV) and hepatitis C virus (HCV) to PEG-IFN plus ribavirin therapy.

METHODS: Fifteen patients infected with HCV were treated with PEG-IFN(0.5 µg/body weight/week) and ribavirin (1 000 mg-1 200 mg/daily) for 48 weeks. Blood samples were drawn at the beginning and the end of the therapy. Serum TTV DNA and HCV RNA were quantified by real time PCR.

RESULTS: At the beginning of treatment, TTV infection was detected in 10/15 (66.6%) of HCV-infected patients. Loss of serum TTV DNA at the end of therapy occurred in 6/10 (60%) patients. Out of these 6 patients, 4 (67%) became positive for TTV DNA after 6 months of therapy. Regarding HCV viremia, 11/15 (73%) patients were negative for serum HCV RNA after 48 weeks of therapy, 7/11 (64%) of these cases also became negative for TTV DNA following the combined treatment. In the 3/4 (75%) patients who were positive for HCV RNA at the end of therapy, TTV DNA was detected as well. Sustained HCV response at 6 months after treatment was 53% (8/15).

CONCLUSION: No TTV sustained response can be achieved in any patient after PEG-IFN plus ribavirin administration.

INTRODUCTION

TT virus (TTV) has been recently identified in patients with elevated alanine aminotransferase (ALT) levels following transfusions[1]. TTV presents an extreme diffusion of active infection throughout the world[2]. It is known that TTV may be highly contagious although its way of spread is poorly understood. However, its high prevalence rate among the people receiving or being in contact with blood products could indicate that TTV can be parenterally transmitted[3]. Although it has been proposed that TT virus be responsible for the small proportion of acute and chronic forms of hepatitis that still remain unsolved, no other illness has yet been attributed to the virus. The TTV genome is a circular, single-stranded DNA of negative polarity, which shares similarities with members of the Circoviridae family and, in contrast to DNA viruses, TTV isolates exhibit a high level of genetic heterogeneity[4,5].

Hepatitis C virus (HCV) is the major cause of chronic liver infection which may progress to cirrhosis and eventually to hepatocellular carcinoma (HCC)[6]. Recently, pegylated interferon (PEG-IFN), initially alone and later in combination with ribavirin, a nucleoside analogue with a broad antiviral activity against a variety of DNA and RNA viruses, has provided new perspectives for the treatment of most patients with chronic HCV infection[7,8]. Co-infection of TTV and HCV is commonly seen maybe because both viruses share the same transmission routes such as blood transfusion[9]. In previous studies, IFN therapy was reported to be effective against TTV[10,11], but the possible susceptibility of the virus to the combination of PEG-IFN plus ribavirin treatment has not yet been investigated. Thus, we investigated the response of TTV infection to PEG-IFN plus ribavirin therapy in patients with chronic hepatitis C and evaluated whether PEG-IFN plus ribavirin combined therapy on chronic hepatitis C was influenced by a TTV co-infection.

MATERIALS AND METHODS

Patients

We enrolled randomly in the study 15 patients (11 males and 4 females, mean age: 41.6 years, range: 30 to 57 years) with chronic HCV infection who had undergone PEG-IFN plus ribavirin therapy. The diagnosis of chronic hepatitis was made on histological (stage of fibrosis and grade of activity) and biochemical liver function tests. Five patients had a history of blood transfusion, 6 acquired HCV infection by parenteral route (intravenous drug abusers, tattoos...) and in 4 patients the transmission route was unknown. Biochemical and virological features of the patients are shown in Table 1.

PEG-IFN was administered intramuscularly at a dose of 0.5 µg/body weight/week, ribavirin was given orally at a dose of 1 000-1 200 mg/daily (weight adjusted) for 48 weeks. Blood samples were taken at the baseline time and when therapy was stopped. To evaluate the effects of PEG-IFN plus ribavirin, levels of ALT, TTV DNA and HCV RNA were evaluated at each time. TTV and HCV clearance was defined as the disappearance of serum TTV DNA and HCV RNA after 48 weeks of combined treatment. All patients gave written informed consent before enrollment in the study, which was approved by the Ethics Committee of the hospital.

Detection and quantification of TTV DNA

Total DNA was purified from 200 µl of serum using the high

pure viral nucleic acid kit (Roche Diagnostic, Mannheim, Germany) and eluted in 50 μl distilled water. TTV DNA was subjected to nested PCR for qualitative analysis. First PCR was performed with primer pair NG054/NG132[12] and nested PCR with primer pair T801/T935[13]. In those positive samples for viral DNA, TTV DNA quantification was carried out with real time PCR by the SYBR Green approach using primers targeting a fragment of the untranslated region (UTR) of the viral genome as previously described by Garcia *et al*[14].

HCV markers
HCV antibodies (anti-HCV) were determined by immunoassay (Ortho Diagnostic System, Raritan, NJ). Serum HCV RNA levels were measured using the Amplicor HCV monitor test (Roche). HCV genotyping was carried out using the Inno-Lipa HCV test (Innogenetics).

Statistical analysis
Statistical analyses were performed using Student' s *t* test. Data were analyzed with the computer program SPSS (SPSS Inc., Chicago, IL, USA). A probability (*P*) value less than 0.05 was considered statistically significant.

RESULTS

Detection and response of TTV to PEG-IFN plus ribavirin therapy
Fifteen patients infected with HCV were monitored randomly for levels of TTV and HCV in serum, at the beginning and end of PEG-IFN plus ribavirin combined treatment. Of the 15 patients, serum TTV DNA could be detected in 10/15 (67%) by real time PCR at the beginning of therapy, with a TTV value that ranged from 1.3×10^3 to 1.7×10^5 genomes/ml of serum (mean: 3.4×10^4 genomes/ml). After 48 weeks of PEG-

IFN plus ribavirin therapy, 6/10 patients (60%) lost serum TTV DNA. Regarding the 4 patients who still had detectable serum TTV DNA, TTV value ranged from 10^3 to 4×10^4 genomes/ml of serum (mean: 1.4×10^4 genomes/ml). In 3/4 patients (75%) positive for TTV, circulating HCV RNA was detected simultaneously after completion of the combined therapy. With respect to these 4 positive TTV DNA patients, at the end of treatment and relative to baseline levels, 3 patients (75%) had a reduction of serum TTV load and 1 case (25%) presented an increase in the levels of serum TTV DNA. The latter patient presented a grade III fibrosis while the remaining 3 individuals presented a grade I fibrosis (Table 1).

When TTV DNA was analyzed at 6 months after stopping PEG-IFN plus ribavirin administration, four responders (67%) had a relapse of TTV viremia, in the 2 remaining cases no serum samples were available (Table 1). The 4 patients, who did not eliminate TTV DNA at the end of treatment, still maintained DNA during the follow-up period. With respect to the 5 TTV DNA negative patients at the beginning of therapy, 1 (20%) became positive for TTV after stopping combined therapy, 2 (40%) cases remained negative for TTV and in 2 individuals viral marker was not determined (Table 1).

Serum TTV DNA level with respect to HCV in non-responder and responder patients to PEG-IGN plus ribavirin treatment was also analyzed and no statistically significant differences were found when pretreatment serum samples were compared between both groups (*P*=0.281). However, we observed that those patients eliminating TTV at the end of therapy presented a lower basal HCV load when compared with the non-responder TTV patients (1.1×10^6 *vs* 2.1×10^6 genomes/ml, respectively *P*=0.03). Regarding the changes of ALT levels, no statistical differences were observed when they were analyzed between TTV responder and non-responder (Table 2). In contrast, serum ALT level was persistently maintained

Table 1 Features of patients in the study

Patient No.	HCV Genotype	Fibrosis	Before treatment			End of treatment			6 months after treatment		
			ALT	TTV	HCV	ALT	TTV	HCV	ALT	TTV	HCV
1	1a	I	51	1.7×10^5	5×10^5	26	-	-	83	+	ND
2	1a	III	139	1.5×10^3	9.4×10^5	35	-	-	19	ND	-
3	1a	III	102	1.7×10^3	8×10^5	30	-	-	11	+	-
4	1b	III	225	1.1×10^4	1.1×10^6	29	4×10^4	-	27	+	-
5	1a	II	82	1.4×10^3	9×10^5	30	-	-	168	+	1.9×10^6
6	1b	III	92	1.3×10^3	1.5×10^6	42	-	-	48	+	-
7	3a	II	117	1.8×10^4	2.4×10^6	9	-	-	23	ND	-
8	1b	III	102	-	1.5×10^6	59	-	-	39	-	-
9	1b	I	86	5.7×10^4	7×10^5	30	1.3×10^4	6×10^5	44	+	9.9×10^5
10	1b	I	183	-	9×10^4	26	-	-	17	+	-
11	1a	I	77	-	2.5×10^6	16	-	-	27	ND	-
12	1b	I	72	4.6×10^4	2×10^5	36	10^3	3.5×10^5	76	+	5×10^5
13	1a	III	101	-	2.3×10^5	47	-	3×10^4	153	ND	+
14	1b	I	68	-	5×10^5	29	-	-	56	-	2×10^5
15	1a	I	29	3.1×10^4	6.3×10^6	13	2.5×10^3	2.1×10^6	30	+	+

ALT level was expressed as IU/L. TTV and HCV loads were expressed as viral genomes/ml of serum.

Table 2 Changes of ALT levels in TTV and HCV responder and non-responder patients to PEG-IFN plus ribavirin therapy

	TTV		P[a]	HCV		P[a]
	Responders (*n*=6)	Non-responders (*n*=4)		Responders (*n*=11)	Non-responders (*n*=4)	
Before therapy	97.16±30.18	103.0±84.87	0.09	112.54±51.90	72.00±31.01	0.33
End of therapy	28.66±11.09	27.00±9.83	0.92	30.09±12.96	31.50±14.20	0.76

ALT level was expressed as IU/L. [a]Student´s *t*-test.

in the normal range before and after the combined therapy in one patient who did not eliminate TTV and HCV (Table 2, patient No. 15).

HCV response to PEG-IFN plus ribavirin treatment

Pretreatment serum HCV RNA values ranged between 9×10^4 and 6.3×10^6 genomes/ml of serum (mean: 1.36×10^6 genomes/ml). The decline of HCV viremia was clearly evident after therapy and the response rate was 73% (11/15). When serum HCV RNA pretreatment levels were compared between non-responder and responder patients, the difference was statistically significant (1.85×10^6 *vs* 1.15×10^6 genomes/ml, respectively; $P=0.005$). Sustained HCV response after 6 months of treatment was found in 8/15 (53%) patients, 2 patients (13%) became HCV RNA positive during the follow-up period and in one case no serum sample was available for the detection of HCV RNA (Table 1). Following combined therapy, baseline and final ALT levels between responder and non-responder patients were compared but the differences between both groups were not statistically significant (Table 2).

DISCUSSION

Many studies have been done trying to assess whether TTV could cause liver disease, but its molecular properties and its pathogenic potential are still poorly understood. Different epidemiological studies have clearly indicated that TTV is a transmissible blood-borne virus sharing common transmission routes with hepatitis viruses. Then, coinfection of TTV was frequently observed in patients with chronic hepatitis C[9]. We found that 66.6% of our patients infected with HCV were TTV DNA-positive, which confirms previous studies showing that the prevalence of TTV infection varied between 30%-88%[15,16].

It has been reported that TTV infection treated with IFN alone had a response rate of 62%-83% after monotherapy[11,17]. Our data showed that 60% of the TTV-infected patients could eliminate TT virus at the end of PEG-IFN plus ribavirin combination treatment. By comparing our results with earlier published studies, the novel and interesting information obtained from our work is that the treatment of TTV infection with PEG-IFN plus ribavirin, for a period of 48 weeks, did not promote an additional and increased response to therapy. Then, ribavirin did not produce any effect on TTV replication because of the similar sensitivity of TTV to combined treatment or to standard IFN monotherapy. Moreover, in these published studies the sustained TTV response after 6 months of therapy was decreased to 32%-50%[18]. In contrast, in our series no sustained clearance of TTV was achieved in any patient after 6 months of combined therapy. It is well known that TTV tended to last many years[19] although spontaneously resolved viremias after short periods of time were described in the literature[20]. That the majority of our TTV responder patients relapsed during the follow-up period could suggest the possibility that TTV could become temporarily latent, as a result of the sensitivity of TTV to PEG-IFN during its administration, and that a new reinfection of the virus occurred. In other words, it was possible that TTV could persist in other type cells such as peripheral blood mononuclear cells where TTV persistence could occur[10]. Among other explanations, it could be possible that because of TTV high level of genetic diversity, viral genomes with different PEG-IFN sensitivities could arise over time. Finally, we suggest that TTV response to PEG-IFN may be affected by a combination of virus and host factors as observed in other viruses[21].

With respect to the effect of PEG-IFN plus ribavirin administration for the treatment of chronic hepatitis C, this study confirms earlier reports in which HCV infection responded positively to the combined therapy with a high rate of viral clearance and normalization of ALT levels during the treatment period. Both factors occurred in HCV/TTV-coinfected patients as well as in non-coinfected ones, so it could be suggested that the response of HCV to IFN-PEG plus ribavirin treatment was not affected by TTV coinfection. Moreover, in those patients without sustained TTV response ALT levels were normal in contrast with those who had a HCV relapse. Then, in agreement with other published studies, TTV might not have any clinical association with producing HCV hepatitis[16,22]. Furthermore, another interesting result from our study was that PEG-IFN plus ribavirin therapy seemed to be specially efficacious in patients with more advanced liver diseases such as grade III fibrosis. Thus, in 45.5% of HCV responder patients with advanced fibrosis, only 25% of non-responder ones presented a minor grade fibrosis, which supported the beneficial effect of PEG-IFN on patients with difficult-to-treat diseases reported in previous studies[23,24].

In conclusion, TTV has a similar response rate to PEG-IFN plus ribavirin combined treatment or to IFN monotherapy, suggesting that neither PEG-IFN nor ribavirin has any additional effect on TTV replication. Furthermore, TTV relapse was found in most of the responder patients after the combined therapy was stopped for 6 months, so it seems likely that TTV is not as sensitive to PEG-IFN as HCV.

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