Influence of TT Virus Co-infection on IFN-β Therapy in Patients with Chronic Hepatitis C

Toshihiro YAMADA1)2), Hirotaka NAITOU2) & Tamotsu MORITA2)

1)Department of Clinical Laboratory Shizuoka General Hospital
2)Laboratory of Environmental Microbiology. Graduate School of Nutritional and Environmental Sciences, University of Shizuoka

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Abstract

Although results of IFNα therapy in chronic hepatitis C (C-CH) patients co-infected with TT virus (TTV) have been reported, no results of IFN β therapy or IFN β and α combination therapy have been reported. In this study, we retrospectively investigated whether co-infection with TTV affects the results of IFN therapy by using stored sera from 60 C-CH patients co-infected with TTV who underwent IFN β therapy or IFN β and α combination therapy. The stored sera were from 29 complete responders, 10 incomplete responders, and 21 non-responders, and they were used for qualitative and quantitative analysis of HCV RNA, HCV genotype analysis, and qualitative and quantitative analyses of TTV DNA. TTV DNA was detected in 23 (38.3%) of the 60 C-CH sera. The TTV DNA-positive rate was 17.2% among the complete responders to IFN therapy, versus 58.1% in the incomplete responders and non-responders, and the difference was significant (p<0.01). While the complete response prediction rate based on two factors, HCV RNA level and HCV genotype, was 80.8% (21/26) in the C-CH patients, the prediction rate based on three factors, these two factors plus TTV DNA, was higher, 90.0% (18/20). It was concluded that determination of HCV RNA concentration, HCV genotype, and TTV DNA, before IFN β therapy or IFN β and α combination therapy is useful for predicting the results of treatment of C-CH patients.


Introduction

Nearly all the factors that determine the results of IFN therapy in chronic hepatitis C (C-CH) patients have been identified1). HCV genotype and HCV RNA concentration are known to be factors that are closely related to the efficacy of IFN therapy2). The most common HCV genotypes in Japanese patients are types 1b, 2a, and 2b3), the efficacy of IFN therapy has been reported to be low for genotype 1b, but high for types 2a and 2b4). The efficacy of IFN therapy increases as the HCV RNA concentration declines5~8). A second course of IFN is now being given to C-CH patients in Japan in whom the first course of IFN therapy does not improve the symptoms, and there has been a desire for a new predictive factor...
for the outcome of therapy other than HCV genotype and HCV RNA concentration.

TT virus (TTV) was recently isolated by Nishizawa et al.\(^9\) from the serum of patients with non-A-G posttransfusion hepatitis by the representational difference analysis (RDA) method\(^10\). Although TTV is a DNA virus, it undergoes many mutations, and many genotypes exist\(^11\)–\(^13\). TTV type 1a has been reported to possess the same genotype as the prototype TTV strain (TA278 strain), and TTV may be a hepatitis virus, because TTV type 1a levels have been found to be highly correlated with changes in ALT in patients with acute posttransfusion hepatitis\(^8\). The TTV detection rate with N22 primer pairs, which detect all genotypes, including type 1a, was 12% in healthy volunteers, and significantly higher in C-CH patients, ranging from 33.3% to 40.0%\(^14\). However, although the results of IFN \(\alpha\) therapy of chronic hepatitis C (C-CH) patients co-infected with TTV have been reported\(^15\)–\(^16\), the results of IFN \(\beta\) therapy or IFN \(\beta\) and \(\alpha\) combination therapy\(^17\) have not been reported. In this study, we retrospectively investigated whether co-infection with TTV affects the results of IFN therapy by using stored sera from C-CH patients with TTV co-infection who underwent IFN \(\beta\) therapy or IFN \(\beta\) and \(\alpha\) combination therapy.

**Materials and Methods**

**Patients**

Frozen sera RNase contamination was avoided, and the sera were stored at \(-80^\circ\text{C}\) collected from 60 C-CH patients before and after IFN \(\beta\) therapy or IFN \(\beta\) and \(\alpha\) combination therapy were used. Patient age ranged from 17 to 68 years old (mean: 43.6 years old), and there were 46 males and 14 females. Twenty-four patients underwent IFN \(\beta\) therapy (6 \(\times\) 10^6 unit IFN \(\beta\), daily for eight weeks) and 36 patients underwent combination therapy with IFN \(\beta\) and \(\alpha\) (6 \(\times\) 10^6 unit IFN \(\beta\), daily for four weeks, followed by 6 \(\times\) 10^6 unit IFN \(\alpha\), three times a week for 20 weeks).

**Evaluation of the therapeutic efficacy of IFN in C-CH patients**

To judge the efficacy of IFN therapy, patients were classified as complete responders (CRs), incomplete responders (ICRs), and non-responders (NRs) according to the criteria below. CRs were defined as patients in whom the ALT value became normal within six months after the end of IFN administration and persisted for six months or longer at a normal level and in whom the HCV RNA-negativity detected by the polymerase chain reaction (PCR) also persisted. ICRs were defined as patients in whom the ALT value became normal within six months after the end of IFN administration and persisted at a normal level for six months or longer, but in whom HCV RNA detected by PCR did not convert to negative. NRs were defined as patients whose ALT values did not become normal within six months after the end of IFN administration and in whom HCV RNA detected by PCR did not convert to negative.

**Qualitative, quantitative analysis, and genotype analysis of HCV RNA**

HCV RNA in stored sera collected before IFN therapy was quantified with an AMPLICOR HCV MONITOR version 2.0 kit (Roche Diagnostics Co., Ltd., Tokyo, Japan). HCV in stored sera collected before IFN therapy were genotyped, were typed as 1a, 1b, 2a, or 2b with an SMI TEST HCV genotype kit (Genome Science Co., Ltd., Tokyo, Japan). For qualitative analysis of HCV RNA, stored sera collected at the end of IFN therapy and six months and one year after the end of the therapy were tested with an AMPLICOR HCV Detection kit (Roche Diagnostics, Co., Ltd., Tokyo Japan).
Qualitative and quantitative analysis of TTV DNA

TTV DNA was qualitatively and quantitatively analyzed by semi-nested PCR using primer pairs for the N22 region. The outer primers were NG059 (5'-CAG ACA GAG GAG AAG AAG GCA ACA TG-3') and NG063 (5'-CTG GCA TTT TAC CAT TTC CAA ATG T-3'), and the inner primers were NG061 (5'-GGC AAC ATG TTA TGG ATA GAC TGG-3') and NG06338. To quantify the TTV DNA, viral DNA extracted from patient serum collected before IFN therapy was serially diluted 10⁻¹⁰⁵ times and subjected to PCR with the N22 primer pairs.

A Gene Amp PCR system 9600-R (Perkin Elmer Co., Ltd., Tokyo Japan) was used as the gene amplification apparatus. The PCR program for detection of TTV DNA was as follows. In the 1st PCR, after heating at 95°C for 10 minutes, the DNA sample was subjected to 35 cycles of reactions consisting of heating at 94°C for 20 sec, 55°C for 20 sec, and 72°C for 30 sec, and then heated at 72°C for 7 min. In the 2nd PCR, 5.0 µl of the amplified product obtained by the 1st PCR was heated at 95°C for 10 min, then subjected to 35 cycles of reactions consisting of heating at 94°C for 20 sec, 60°C for 20 sec, and 72°C for 30 sec, and then heated at 72°C for 7 min. After PCR, the products were electrophoresed at 100 V, 80 mA for 30 minutes on a 3% agarose gel containing 0.5µg/ml ethidium bromide. After electrophoresis, the gel was photographed under UV illumination, and 286 bp and 271 bp bands were amplified in the 1st and 2nd PCR, respectively.

Statistical analysis

Statistical analysis of the differences in serum HCV RNA concentrations in the CRs, ICRs and NRs was performed by Student’s t-test (two-sided). Statistical analysis of the differences in serum TTV DNA-positive rates in the CRs, ICRs and NRs was performed by the χ²-test. Correlations between serum HCV RNA concentrations and serum TTV DNA concentrations were tested using by Spearman’s rank correlation coefficient. Statistical analysis of the relevance in serum HCV RNA concentration and TTV DNA concentration was performed by the χ²-test. Multivariate logistic regression analysis was used to analyze the contribution of TTV infection to the response of HCV to IFN therapy. HCV-RNA concentration in serum, HCV-genotype, and TTV infection were selected as explanatory variables. In all the statistical analyses, P values less than 0.05 were considered significant.

Results

Prediction rate of IFN therapeutic effect

Of the 60 C-CH patients who underwent IFN β therapy or IFN β and α combination therapy, 29 patients (48.3%) were CRs. The Complete response rate was 41.7% (10/24) for IFN β therapy and 52.8% (19/36) for combination therapy. The HCV RNA concentration of the CRs before IFN therapy was 221.7 ± 357.6 kilo international unit (KIU)/ml, as opposed to 1,086.0 ± 1,662.0 KIU/ml in the ICRs and NRs, showing a significant difference between the two groups (p< 0.01). HCV genotyping in all 60 sera showed that it was 1b in 31, 2a in 21, 2b in 8, and a mixed type (2a and 2b) in 1. Among the patients with TTV genotype 1b, 7 were CRs and 24 were ICRs or NRs. Among the other genotypes, 22 patients were CRs and 7 were ICRs or NRs.

Of the 60 C-CH patients, 23 were TTV DNA-positive before IFN therapy, and the positive rate was 38.3%. The clinical and virologic characteristics of the 23 patients with TTV infection and 37 patients
Table 1 Comparison of the characteristics of TTV Infection-positive and -negative chronic hepatitis C patients

<table>
<thead>
<tr>
<th>Background</th>
<th>TTV infection</th>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n=23)</td>
<td>Negative (n=37)</td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>45.2 ± 122</td>
<td>42.7 ± 137</td>
<td>0.238**</td>
</tr>
<tr>
<td>Sex (% males)</td>
<td>87.0</td>
<td>70.3</td>
<td>0.205***</td>
</tr>
<tr>
<td>HCV genotype-positive patients (%)</td>
<td></td>
<td></td>
<td>0.111***</td>
</tr>
<tr>
<td>1a/1b</td>
<td>15 (65.2)</td>
<td>16 (43.2)</td>
<td></td>
</tr>
<tr>
<td>2a/2b</td>
<td>8 (34.8)</td>
<td>21 (56.8)</td>
<td></td>
</tr>
<tr>
<td>Median (range) HCV RNA (KIU/ml*)</td>
<td>619 (6-2,604)</td>
<td>699 (1-8,791)</td>
<td>0.490****</td>
</tr>
<tr>
<td>IFN-β only (%)</td>
<td>9 (39.1)</td>
<td>14 (37.8)</td>
<td>1.000***</td>
</tr>
</tbody>
</table>

* KIU/ml: kilo international units/ml, ** Student's t-test, *** Chi-squared test, **** Mann-Whitney U-test

Table 2 Relationship between response to IFN and TTV infection in C-CH

<table>
<thead>
<tr>
<th>TTV infection</th>
<th>Positive (n=23)</th>
<th>Negative (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR (%)</td>
<td>5 (17.2)</td>
<td>24 (82.8)</td>
</tr>
<tr>
<td>ICR and NR (%)</td>
<td>18 (58.1)</td>
<td>13 (41.9)</td>
</tr>
</tbody>
</table>

TTV, TT virus; C-CH, chronic hepatitis C; CR, complete responder; ICR, incomplete responder; NR, non responder.

*p < 0.01 (relevance of IFN effect and TTV infection).

Table 3 Multivariate logistic regression analysis of factors possibly correlated with the hepatitis C virus response to IFN-β therapy

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Logistic regression coefficient</th>
<th>SEM</th>
<th>R statistic</th>
<th>Wald statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV RNA concentration</td>
<td>-0.544</td>
<td>0.223</td>
<td>-0.219</td>
<td>5.952</td>
<td>0.014</td>
</tr>
<tr>
<td>HCV RNA genotype</td>
<td>-1.834</td>
<td>0.766</td>
<td>-0.212</td>
<td>5.736</td>
<td>0.017</td>
</tr>
<tr>
<td>TTV infection</td>
<td>-1.779</td>
<td>0.732</td>
<td>-0.217</td>
<td>5.901</td>
<td>0.015</td>
</tr>
</tbody>
</table>

without TTV infection are compared in Table 1. Mean age, sex ratio, HCV genotype, HCV RNA concentration, and IFN-β ratio did not differ between the two groups.

The TTV-DNA-positive rate was 17.2% in the CRs, as opposed to 58.1% in the ICRs and NRs, showing a significant difference between the two groups (p < 0.01) (Table 2). Multivariate logistic regression analysis was used to identify factors that contributed to the complete response of HCV to IFN therapy. The results showed that TTV infection was a significant factor, and HCV RNA concentration and HCV genotype were also significant (Table 3).

Twenty-six of the 60 C-CH patients met the following criteria: HCV RNA concentration before IFN therapy no more than 100 KIU/ml when the genotype was 1b and no more than 300 KIU/ml when the genotype was 2a, 2b; 21 of them were CRs, and the Complete response prediction rate was 80.8%.
Fig. 1 Prediction of IFN therapeutic effect by quantity of HCV RNA and HCV Genotype and TTV DNA

Numbers in parentheses indicate the number of complete responders.

* HCV RNA concentrations before IFN therapy no greater than 100 KIU/ml for genotype 1b and no greater than 300 KIU/ml for genotypes 2a, 2b.

Table 4 Relationship between quantity of HCV RNA and quantity of TTV DNA in the serum of C-CH patients co-infected with TTV

<table>
<thead>
<tr>
<th>Quantity of TTV DNA (titer)</th>
<th>Quantity of HCV RNA (KIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;100 (n = 4)</td>
</tr>
<tr>
<td>10^1 (n = 14)</td>
<td>1</td>
</tr>
<tr>
<td>10^2 (n = 6)</td>
<td>2</td>
</tr>
<tr>
<td>10^3 (n = 3)</td>
<td>1</td>
</tr>
</tbody>
</table>

HCV, Hepatitis C virus; TTV, TT virus; C-CH, chronic hepatitis C; TTV DNA quantity, there was no cases with levels of 10^4 or 10^5.

*p > 0.05 (relevance of quantity of HCV RNA and quantity of TTV DNA).

Twenty patients met the criterion: TTV DNA-negative before IFN therapy in addition to the above criteria: 18 of them were CRs, and the Complete response prediction rate was 90.0% (Fig. 1).

**Correlation between HCV RNA concentration and TTV DNA concentration**

The HCV RNA concentrations and the TTV DNA concentrations were compared in 23 sera in which TTV DNA was detected, but the correlation coefficient was 0.089, showing no correlation. The HCV RNA concentrations and the TTV DNA concentrations were divided into three classes: low, medium, and high, and the independence of the frequency table was tested, but no significant difference was obtained (p > 0.05) (Table 4).

**Discussion**

Since results of this study showed no inverse correlations between the virus concentrations of patients co-infected with HCV and TTV. There may be no or a very weak viral interference phenomenon between HCV and TTV, and HCV replication may not be inhibited in C-CH patients with TTV co-infection. The Complete response rate after IFN β therapy and IFN β and α combination therapy was significantly higher in C-CH patients without TTV co-infection than in C-CH patients with TTV co-infection. Based on the above, TTV co-infection may be a negative factor for therapeutic efficacy in C-CH patients. By contrast, TTV co-infection has been reported to have no effect on the efficacy of IFN-α therapy. Although the reason for the discrepancy between those reports and our study is unclear, inhibition of 2'-5'-oligoadenylate synthetase (2-5 OAS) activity in cells co-infected with HCV and TTV is considered to be the cause of the effect of TTV on IFN therapy in C-CH patients. HCV is an RNA virus, and the antiviral effect of IFN is due to degradation of viral genomic RNA by the 2-5 OAS activation mechanism in HCV-infected cells. However, in cells co-infected with HCV and TTV, TTV may have affected the IFN thera-
peutic efficacy of IFN by inhibiting 2-5 OAS activity and preventing degradation of HCV RNA.

In C-CH patients meeting the two clinical useful criteria: HCV RNA concentration no greater than 200 KIU/ml and non-1b HCV genotype, the Complete response prediction rate was 84.2%. When TTV DNA negativity was added as a new factor for predicting efficacy, the Complete response prediction rate increased to 93.3%, showing that presence or absence of TTV co-infection may be a useful factor for predicting the efficacy of IFN β therapy and IFN β and α combination therapy in C-CH patients. Moreover, a multivariate logistic regression analysis indicated that TTV co-infection, HCV RNA concentration, and HCV genotype were significant predictive factors of therapeutic efficacy. Therefore, it was concluded that these three factors: HCV RNA concentration, HCV genotype, and TTV DNA, are useful for predicting the therapeutic efficacy of IFN β therapy and IFN β and α combination therapy. Prediction of therapeutic efficacy by these three factors will be effective in selecting C-CH patients who will respond to IFN β therapy and IFN β and α combination therapy.

Acknowledgements

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References

Influence of TTV co-infection with C-CH

abstract


C 型慢性肝炎患者のインターフェロン β 療法における TT virus 重感染の影響

静岡県立総合病院検査部 1, 静岡県立大学大学院生活健康科学研究科 2
山田 優博 1,2 内藤 博敬 2, 森田 全 2

TT virus（TTV）を重感染する C 型慢性肝炎（C-CH）患者のインターフェロン（IFN）療法に関しても、これまで IFNα療法の治療成績は報告されているものの、IFNβ療法や IFN βα 併用療法の治療成績については末だその報告は認められていない。今回我々は、IFN β療法または IFN βα 併用療法を施行された TTV 重感染の C-CH 患者の保存血清を用いて、TTV の重感染が IFN の治療効果に影響を与えていたか否かについて retrospective に検討した。IFN β療法または IFN βα 併用療法を施行された C-CH 患者 60 例 [治療効果の内訳は、Complete responder (CR) が 29 例、Incomplete responder (ICR) が 10 例、no responder (NR) が 21 例] の保存血清を用いて、HCV RNA の定量と TTV DNA の定量を行った。C-CH 患者 60 例中 23 例（38.3%）に TTV DNA が検出された。IFN 治療の CR においては、その TTV DNA 陽性率が 17.2% であったのに対し、ICR および NR では 58.1% であり、両群間には有意差が認められた (p<0.01)。さらに、HCV RNA 量、HCV genotype の 2 つの因子からみた C-CH 患者の IFN 治療効果の CR 予測率が 80.8% (21/26) であったのに対し、TTV DNA を加えた 3 つの因子からみた CR 予測率は 90.0% (18/20) と高率であった。C-CH 患者において、HCV RNA 量、HCV genotype および、TTV DNA の 3 因子を IFN β療法または IFN βα 併用療法の治療前に測定する事は、その治療効果の予測に有用であると考えられた。

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