Hepatitis C Virus RNA and Anti-N14 Antibody Levels during Interferon Alpha Therapy for Chronic Hepatitis C

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To investigate the markers useful for evaluating the long-term efficacy of interferon (IFN) therapy, the quantity of hepatitis C virus (HCV) RNA and two anti-HCV antibody titers (anti-N14 and anti-C-100-3 antibody) in 21 chronic hepatitis C patients were determined. In all complete responders, a sustained clearance of the virus and reductions in the anti-HCV antibody titers were observed during and after therapy. In most of the temporary responders, reductions in the HCV RNA levels and in both anti-HCV antibody titers were observed temporarily during the therapy, and relapse followed. In nonresponders, although the HCV RNA levels and anti-N14 antibody titer tended to remain unchanged or increased during and after therapy, the anti-C-100-3 antibody titers showed no tendency. These results demonstrate that the monitoring of the HCV RNA level and anti-N14 antibody titer is clinically useful for following the patient’s response to IFN therapy for chronic hepatitis C.

(Key words: multicyclic RT-PCR, anti-HCV antibody, anti-C-100-3 antibody)

Introduction

It has been reported that interferon (IFN) therapy is effective for decreasing the serum alanine aminotransferase (ALT) levels and improving the hepatic histology in patients with chronic hepatitis C (1, 2). However, it has been observed that a recurrence of the hepatitis often occurs after the cessation of therapy even in patients in whom the treatment appeared to be effective (3, 4). Changes in the ALT level are most commonly used for assessing the efficacy of IFN, but the rate of relapse is high in patients with detectable HCV RNA in their serum even when ALT levels become normalized during IFN therapy. Moreover, relapse occurred unexpectedly and often in some patients whose ALT levels normalized after the cessation of therapy. These data suggest that the ALT levels alone do not yield an accurate assessment of the long-term efficacy of IFN. In recent years, an objective evaluation of the anti-viral effect of IFN has become possible by using quantitative analysis of the HCV RNA (5, 6). In addition, it has been reported that the anti-HCV antibody titer is also clinically related to the therapeutic effect of IFN (7, 8), and we have reported that the presence of HCV RNA is closely associated with that of anti-N14 antibody (9). Therefore, to investigate the usefulness of anti-N14 antibody as a convenient monitoring marker in evaluating the long-term efficacy of IFN therapy, we assayed the quantity of HCV RNA and two anti-HCV antibody titers (anti-N14 and anti-C-100-3 antibody) in patients with chronic hepatitis C during IFN therapy, and studied the relationship between these parameters and prognosis of the patients.

Subjects and Methods

Subjects

Twenty-one patients (14 males and 7 females, mean age 48 years, range 23 to 59 years) treated with IFN at Kagoshima Prefectural Hokusatsu Hospital from February to September 1992 were the subjects of this study. Chronic active hepatitis (CAH2A in 14 patients and CAH2B in 7 patients) was histologically diagnosed by liver biopsy (10). A blood transfusion history was present in four patients; one of these was a nurse who had an accidental needle stick injury. All patients were positive for the second generation anti-HCV antibody, but negative for HBs antigen. Anti-nuclear antibody was negative in all patients. Moreover, patients suspected of having alcohol-induced or drug-induced hepatitis were excluded from the study. IFN-alpha-2a (Takeda Chem. Ind. Co., Ltd., Osaka,
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Results

Change in ALT levels during and after therapy

The 21 patients treated were classified as complete responders, temporary responders, and nonresponders according to their ALT levels during and after IFN therapy. A complete response was defined as a decrease in the serum ALT level into the normal range within 6 months after therapy withdrawal (complete responders). In 9 of the 10 complete responders, the ALT level normalized during therapy. In five patients, serum ALT normalization was observed during therapy, and in two patients, a reduction (>50%) in the serum ALT levels was observed during therapy; but in these seven patients an increase in the serum ALT levels occurred after therapy (temporary responders). In the remaining 4 of the 21 patients, serum ALT levels did not change during or after therapy (nonresponders). The clinical, biochemical and virologic data of these three groups are shown in Table 1.

Change in HCV RNA quantity during and after therapy

The changes in the quantity of HCV RNA in these three groups treated with IFN are shown in Figs. 1A, 2A, and 3A. In all complete responders, no HCV RNA was detectable at the end of therapy; it remained undetectable for 6 months after therapy withdrawal. In all temporary responders, the quantity of HCV RNA was decreased by the end of therapy compared to that before therapy, and it was even undetectable in one patient. However, in all these patients the quantity of HCV RNA increased by 6 months after therapy to a higher level than that observed at the end of therapy, and in five patients it was higher than the level before therapy. By contrast, in three of the four nonresponders, the quantity of HCV RNA increased at the end of therapy compared to that before therapy, and in two patient it increased further 6 months after therapy withdrawal compared to that at the end of therapy.

Changes in anti-HCV antibody titer during and after therapy

Changes in the anti-N14 and anti-C-100-3 antibody titers in each group during and after therapy are shown in Figs. 1B, C, and D.

Table 1. Clinical, Biochemical, and Virologic Characteristics of 21 Patients with Chronic Hepatitis C before Interferon Therapy

<table>
<thead>
<tr>
<th></th>
<th>Complete responders (n=10)</th>
<th>Temporary responders (n=7)</th>
<th>Nonresponders (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>6/4</td>
<td>4/3</td>
<td>4/0</td>
</tr>
<tr>
<td>Age (yr (mean±SD))</td>
<td>45.0±13.1</td>
<td>50.1±18.9</td>
<td>51.3±8.5</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>ALT (IU/L (mean±SD))</td>
<td>111.6±128.0</td>
<td>136.7±68.9</td>
<td>96.5±44.5</td>
</tr>
<tr>
<td>Histology (CAH2A/CAH2B)</td>
<td>8/2</td>
<td>4/3</td>
<td>2/2</td>
</tr>
<tr>
<td>HCV RNA level (10^6 copy/ml (mean±SD))</td>
<td>6.4±1.3~b</td>
<td>8.3±1.0</td>
<td>8.5±1.0</td>
</tr>
<tr>
<td>HCV genotype (II:III:IV)</td>
<td>(4.5:1)</td>
<td>(6:0:1)</td>
<td>(4:0:0)</td>
</tr>
</tbody>
</table>

~p<0.01 vs. temporary responders, ~p<0.05 vs. nonresponders.
2B, C and 3B, C. Although second generation anti-HCV antibody was positive in all patients, anti-N14 or anti-C-100-3 antibody was positive in 18 patients before therapy. The change in the second generation anti-HCV antibody titer in a total of nine patients (three patients in each group) was investigated by a preliminary study, but no change was observed in any patient either during or after therapy. The anti-N14 and anti-C-100-3 antibody titers in the complete responders decreased continuously during and after therapy. Both antibody titers in the temporary responders tended to be lower at the end of therapy than before therapy, but they rose again 6 months after therapy withdrawal in all but one patient. In contrast, the anti-N14 antibody titers in the nonresponders either did not change or increased during or after therapy. No definite tendency in the change in the anti-C-100-3 antibody titers was observed. The changes in the anti-N14 and anti-C-100-3 antibody titers compared to those before therapy were determined. A significant decrease in the anti-N14 and anti-C-100-3 antibody levels in the complete responders was observed at the end of therapy and 6 months after therapy withdrawal (p<0.01). In the temporary

![Graphs showing changes in HCV RNA levels and antibody titers](image-url)

**Fig. 1.** Changes in the serum HCV RNA levels (A) and anti-N14 (B) and anti-C-100-3 (C) titers during and after IFN therapy in complete responders. O: patients administered IFN-alpha for 14 weeks; •: patients administered IFN-alpha for 24 weeks.

**Fig. 2.** Changes in the serum HCV RNA levels (A) and anti-N14 (B) and anti-C-100-3 (C) titers during and after IFN therapy in temporary responders. O: patients administered IFN-alpha for 14 weeks; •: patients administered IFN-alpha for 24 weeks.
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Fig. 3. Changes in the serum HCV RNA levels (A) and anti-N14 (B) and anti-C-100-3 (C) titers during and after IFN therapy in nonresponders. O: patients administered IFN-alpha for 14 weeks; ●: patients administered IFN-alpha for 24 weeks.

responders, a significant reduction was observed in the anti-N14 titer at the end of therapy (p<0.05) but not in the anti-C-100-3 titer, nor in either titer 6 months after therapy withdrawal.

Discussion

As shown in the Results, the therapeutic effect of IFN was closely related to changes in the HCV RNA levels and anti-N14 antibody titers. The following two points were clarified by monitoring the HCV RNA levels. First, the clearance of HCV RNA during therapy was the most important finding in the complete responders, because the ALT levels normalized even in temporary responders, and an improvement in the ALT levels was observed even in nonresponders despite increases in their HCV RNA levels. Changes in the ALT level generally, but not always followed the changes in the HCV RNA levels during IFN therapy. This discrepancy may be due to the other effects of IFN itself, such as toxicity against hepatic cells or other immunologic effects (15). Therefore, evaluation of changes in the HCV RNA levels rather than the ALT levels is a better predictor of the long-term prognostic effect of IFN. Next, as observed in the nonresponders, patients who exhibit proliferation of HCV during the administration of IFN should be carefully evaluated. When the virologic background of these patients was investigated, a high HCV RNA level or genotype II was observed (Table 1). The relationship between the genotype or HCV RNA level and histologic grade has been reported as a predictive factor for responsiveness to IFN (16, 17). In addition, in many temporary responders, the HCV RNA level was higher after therapy than that before therapy. This suggests that a long-term anti-viral effect would be expected only in complete responders.

Thus, monitoring HCV RNA levels is useful, but not always reliable for predicting the long-term prognosis of patients treated with IFN for hepatitis C. As shown in one temporary responder in this study (Fig. 2A), temporary disappearance of HCV RNA in the serum during IFN therapy can occur. This might be caused by disappearance of HCV RNA from the serum before the clearance of HCV RNA from the liver by an anti-viral effect of IFN. This same phenomenon was observed more frequently when patients were treated with IFN-beta. Furthermore, monitoring of HCV RNA in clinical laboratories has been delayed because of the technical difficulty and expense of using this PCR assay (18, 19). In this study, we presented a convenient method to monitor the anti-N14 antibody titer, which is simple, inexpensive and can be performed by most clinical laboratories. The change in the anti-N14 antibody titer correlated well with the change in the HCV RNA level. Although the change in the antibody titer was not as sensitive as that in the HCV RNA level, the reduction in the antibody titer may reflect the continuous reduction or clearance of viral antigen in the serum and liver. Therefore, the monitoring of the anti-N14 antibody titer may complement HCV RNA monitoring assay. In the present study, all four cases with a high reduction rate (>50%) of the anti-N14 antibody titer during IFN therapy were complete responders. From the analysis of more patients, we may be able to identify a reduction rate of the anti-N14 antibody titers which correlates with a complete response to IFN therapy. These observations suggest a more effective method of IFN administration. In patients with decreasing HCV RNA levels and anti-N14 antibody titers during IFN administration, IFN should be continued until HCV RNA is cleared from the blood.

Here the change in the anti-N14 antibody titer correlated better with the change in the HCV RNA levels than did with the change in the anti-C-100-3 antibody titer. This discrepancy seems to be due to the fact that the N14 antigen possess only one
epitope (20) and that the anti-N14 antibody level might reflect viral proliferation, as the anti-core antibody (anti-HBc antibody) reflects viral proliferation in chronic hepatitis B infections. In contrast, ALT levels seem to be reflected in the anti-C-100-3 antibody level because a significant correlation between changes in the ALT levels and this antibody level has been observed (21). Among the various core antigens used for the antibody-detection assay, the N14 antigen is the smallest, containing only one epitope. This might be advantageous for increasing specificity.

The present study suggests that monitoring the HCV RNA level and anti-N14 antibody titer during IFN therapy is useful and helpful for evaluating the efficacy and the long-term prognosis of patients with HCV infections undergoing IFN therapy.

References


