Effectiveness of 6-Month Intermittent Administration of Natural Human Interferon-α against Non-A Non-B Chronic Hepatitis

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Key words: chronic hepatitis type non-A non-B (CHNANB), interferon (IFN)

Summary

Interferon (IFN) was administered intermittently for 6 months to the patients with non-A non-B chronic hepatitis (CHNANB), and the effectiveness of the treatment for improving the hepatic function was evaluated. Of 26 patients with CHNANB, 16 received intermittent IFN therapy (IFN group), and 10 were treated by conventional therapies without IFN (non-IFN group). All patients were observed for 1 year. IFN was administered once a day at 3 MU in principle (1 MU in some patients and 6 MU in 1 patient) daily for 1 week immediately after the beginning of the therapy and 3 times a week for the subsequent 6 months at the outpatient clinic. The patients were followed up for at least 6 months after completion of the treatment. In the IFN group, the serum GPT level normalized in 11 (68.8%) of the 16 patients 1 year after the beginning of the treatment. In these 11 patients (normalized group), HCV-RNA was negative or became negative in 3 of the 6 patients in whom the serum HCV-RNA could be examined. Histological grades of inflammation in the liver were also markedly alleviated in the normalized group. The hepatic function did not normalize in any of the 10 patients in the non-IFN group. These findings indicate that IFN therapy is useful for CHNANB.

Introduction

Since Hoofnagle et al. reported that interferon (IFN) is effective for the treatment of non-A non-B chronic hepatitis (CHNANB)⁶, the effectiveness of IFN therapy for the treatment of CHNANB has been gradually established through clinical application at various institutions²,³,⁴. However, no consensus concerning the effects of the therapy has been established yet, and also the optimal dose, the method or the period of IFN administration remains to be clarified.

In this study, we treated patients with CHNANB by the IFN daily for 1 week immediately after the beginning of the therapy and 3 times a week for 6 months, and evaluated the effectiveness of this intermittent IFN therapy for improving the liver function in comparison with conventional non-IFN therapies. The IFN therapy was carried out between July 1988 and March 1991.
Materials and Methods

1) Patients
The subjects consisted of 26 patients who were diagnosed as CHNANB by liver function tests, histological examination of the liver, and evaluation of hepatitis virus markers and who showed persistence of abnormal serum transaminase levels for at least 6 months before the therapy (Table 1).

2) Methods
These 26 patients were divided into two groups, IFN group and non-IFN group. IFN group was consisted of 16 patients who were treated with IFN alone. Non-IFN group was consisted of 10 patients who were treated with conventional medicine such as Stronger Neo-Minophagen C (SNMC) or Shosaikoto only. The subjects were followed up for 1 year.

1. IFN group
(1) Methods of administration
The test drug was interferon-α derived from human lymphoblasts (HLBI, Sumitomo Pharmaceutical). The 3 MU of IFN (1 MU in 2 patients and 6 MU in 1 patient) was administered i.m. once a day daily for 1 week at the beginning of the therapy and 3 times a week for the following 6 months. Most patients were hospitalized for 1 week at the beginning of the therapy.
(2) Observation period
The patients were observed for 12 months from the beginning of the therapy and were followed-up for as long as possible without other drugs.
(3) Evaluation of improvements in the liver function
The liver function was evaluated according to the serum transaminase levels (GOT, GPT: Karmen Unit) determined after IFN administration.

<table>
<thead>
<tr>
<th>Table 1 Background factors</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>No. of patients</td>
</tr>
<tr>
<td>Sex (males:females)</td>
</tr>
<tr>
<td>Age (Mean ± S.D.)</td>
</tr>
<tr>
<td>History of blood transfusion (+)</td>
</tr>
<tr>
<td>Total dose of IFN (MU) Mean ± S.D.</td>
</tr>
</tbody>
</table>

(456MU: 1 case)  
(228MU: 12 cases)  
(76MU: 2 cases)  
(129MU: 1 case)

Histological diagnosis
- PH 1
- CIH 1
- CAH 12
- CAH with LD 2

1) PH: persistent hepatitis  
2) CIH: chronic inactive hepatitis  
3) CAH: chronic active hepatitis  
4) CAH with LD: chronic active hepatitis with lobular disorganization
Effectiveness of Intermittent IFN Therapy

(4) Evaluation of anti-viral effect
Other than the general liver function tests before and after administration, the serum 2-5 AS activity was determined by RIA (Eiken) as an index of the anti-viral effect of IFN. The serum level of β2-microglobulin (BMG), which is the HLA class I antigenic domain, was determined as an index of the T cell cytotoxicity.

(5) Measurement of HCV antibody
HCV antibody was measured before treatment using an ELISA kit (Ortho).

(6) Measurement of HCV-RNA
The serum level of HCV-RNA was determined by the nested polymerase chain reaction (nested-PCR) method using 2 pairs of primers belonging to the 5'-noncoding region of HCV RNA.

(7) Measurement of anti-IFN antibody
Anti-IFN antibody was measured at the end of the therapy by bioassay.

(8) Evaluation of histological improvements
Liver biopsy was performed before the beginning of the IFN therapy and, if possible after the end of the therapy. The histological changes were evaluated according to HAI score, and the degree of improvements was studied.

2. Non-IFN group
This group was treated with Stronger Neo-Minophagen C (SNMC) or shosaikoto at the outpatient clinic and was observed for 1 year.

3. Statistical analysis
Student’s t-test, χ² test, and Mann-Whitney’s U test were used for statistical analysis.

Results

1. Improvements in liver function
Fig. 1 shows the mean value of the serum GPT level in IFN group and non-IFN group, before, during and after IFN therapy. In the IFN group, the serum GPT level was 206.2 ± 243.6 K.U. before the therapy but decreased significantly (p<0.05) after the beginning of the therapy, becoming 78.8 ± 109.7 K.U. and 43.1 ± 46.2 K.U. (mean ± SD) after 24 and 52 weeks, respectively. No significant improvements were noted in the non-IFN group during the same period.

The serum GPT level normalized (serum GPT≤35 K.U.) during IFN therapy in 12 out of the 16 patients, and in 8 of these 12 patients it remained normal for more 6 months until the end of the therapy.
Fig. 2 Classification of patterns of sGPT changes in IFN group

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Decreased rapidly after beginning of therapy and remained normal after the end of administration</td>
<td>8/16 (50.0%)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Serum GPT temporarily elevated during therapy but normalized after end of therapy</td>
<td>1/16 (6.3%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Serum GPT decreased during IFN therapy, elevated temporarily after the end of therapy, but decreased again to normal</td>
<td>2/16 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Serum GPT decreased during IFN therapy, but elevated after end of therapy, and did not improve</td>
<td>2/16 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>No significant improvement was observed during or after IFN therapy</td>
<td>3/16 (18.8%)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3 Changes of sGPT levels and HCV-RNA in the type I patient

observation period. It elevated again in the remaining 4 during or after the therapy but normalized again and remained normal until the end of the observation period in 2 of these 4 patients. In 1 patient, the serum GPT level elevated during the therapy, but it decreased gradually to a normal level and remained normal for more than 6 months until the end of the observation period. Thus, the serum GPT level was normalized and remained normal in 11 of the 16 patients treated with IFN. No normalization of the serum GPT level was observed in any of the 10 patients of non-IFN group.

The patterns of changes in the serum GPT level in IFN group could be classified into 5 types (Fig. 2). In type I, the serum GPT level decreased rapidly after the beginning of the therapy and remained normal after the end of the administration. In type II, serum GPT temporarily elevated during the therapy but normalized after the end of the therapy. In type III, the serum GPT level decreased during IFN therapy, elevated temporarily after the end of the therapy, but decreased again to normal. In type IV, the serum GPT level decreased during IFN therapy, but elevated after the end of the therapy, and did not improve. In type V, no significant improvement was observed during or after IFN therapy.

Changes in the serum GPT level in each patient of IFN group are shown in Figs 3-7 separately for types I-V.

In 8 of the 16 patients, the serum GPT level decreased rapidly after the beginning of INF therapy and remained normal until 6 months after the end of the administration (Type I, Fig. 3). In 1 patient, the administration was discontinued 13 weeks from the beginning, because the serum GPT level elevated
after the beginning of the administration and remained high level. In this patient, the serum GPT level decreased gradually after discontinuation of IFN administration to a normal level and remained normal 6 months after the end of the therapy (Type II, Fig. 4). In 4 patients, the serum GPT level normalized at least temporarily during the therapy but elevated again. In 2 of these patients, it decreased again and became normal 6 months after the end of the administration (Type III, Fig. 5). In the other 2, it elevated again and did not normalize despite slight improvements (Type IV, Fig. 6). In the remaining 3 patients, the serum GPT level did not normalize during or after the therapy (Type V, Fig. 7).
2. Changes in the serum HCV-RNA level

Figs. 3-7 show changes in the serum level of HCV-RNA determined in 9 patients treated with IFN. HCV-RNA became negative after treatment in 2 of the 4 patients with type I changes in the serum GPT level, but it remained positive in the other 2 patients. In 1 patient with type II changes in the serum GPT level, HCV-RNA remained negative after IFN therapy, but HCV antibody was positive. In 1 patient who showed type III changes in the serum GPT, HCV-RAN remained positive. In 1 of the 2 patients who showed type IV changes in the serum GPT level, HCV-RNA became negative when the serum GPT normalized during IFN therapy, but it reappeared when the serum GPT elevated after IFN therapy.

HCV-RNA remained positive in the other patient who showed type IV changes in the serum GPT level and 1 patient who showed type V changes.

3. Changes in serum 2-5 AS activity

Fig. 8 shows changes in the serum 2-5 AS activity in 11 patients in whom the serum GPT level normalized (Type I, II, or III; normalized group) and 5 patients in whom it did not normalize (Type IV or V; non-normalized group). The serum 2-5 AS activity (mean ± SD) was 93.5 ± 79.5 pmol/dl in the normalized group and 54.3 ± 46.1 pmol/dl in the non-normalized group immediately after the beginning of the therapy. One week after the beginning of the administration, it increased to 165.8 ± 141.0 pmol/dl and 78.3 ± 27.5 pmol/dl, respectively, being significantly higher in the normalized group (p<0.05). It became 101.2 ± 40.3 pmol/dl and 118.0 ± 58.9 pmol/dl after 12 weeks, and 99.9 ± 53.8 pmol/dl and 80.0 ± 19.7 pmol/dl, respectively, after 24 weeks.

4. Changes in the serum BMG level

Fig. 9 shows changes in the serum BMG level in the 11 patients of the normalized group and the 5
Effectiveness of Intermittent IFN Therapy

Fig. 9 Changes of serum $\beta_2$-Microglobulin levels in normalized and non-normalized groups (Mean ± S.D.)

Fig. 10 Changes in the total HAI score in the normalized group after IFN therapy

Serum BMG increased slightly during IFN therapy, but it gradually decreased after the end of the therapy in the normalized group while it remained increased in the non-normalized group.

5. Histological improvements

Fig. 10 shows changes in the total HAI score in the 4 patients of the normalized group who underwent liver biopsy before and after IFN therapy. The total HAI score (mean ± SD) was 9.3 ± 5.0 before the therapy and 1.5 ± 1.3 after the therapy, showing a significant decrease after the therapy. HAI score showed significant improvements in categories I (periportal +/- bridging necrosis) and III (portal inflammation).

6. A patient followed up for a long period

Fig. 11 shows changes in the serum transaminase levels in a patient who was followed up for a long period. The liver function was completely normal 12 months after the end of the therapy. In this patient, the cut-off value of HCV antibody was recently reduced to 1.0. Fig. 12 shows histological profiles of the liver of this patient before the therapy, 2 months after the end of the therapy, and 13 months after the end of the therapy. Before the therapy, lobular remodeling and piecemeal necrosis were noted, and inflammatory cell infiltration was remarkable in the portal region. The histological profile markedly improved after treatment, with few infiltrating cells being noted and fibrosis having disappeared.

平成4年6月20日
Fig. 11 Changes of serum transaminase and C100-3 antibody levels in a patient followed up for a long period

![Graph showing changes in serum transaminase and C100-3 antibody levels](image)

Fig. 12 Liver biopsy specimen taken at before therapy (a), 2 and 13 months after the end of therapy (b, c). (H.E. stain ×400)

The histological profile markedly improved after therapy with few inflammatory cells being noted and fibrosis having disappeared.

(c)

7. Comparisons of the background factors between the normalized and non-normalized groups

Table 2 compares background factors between the normalized group and non-normalized group. No significant differences were noted in any of the factors. However, IFN therapy appeared to be more effective in younger patients without a history of transfusion and with a shorter duration of illness. The presence or
Effectiveness of Intermittent IFN Therapy

Table 2 Patients profiles of normalized and non-normalized groups before IFN therapy

<table>
<thead>
<tr>
<th></th>
<th>Normalized group (n=11)</th>
<th>Non-normalized (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (Mean±S.D.)</td>
<td>36.3±10.4</td>
<td>40.2±9.8</td>
</tr>
<tr>
<td>Sex (males:females)</td>
<td>8:3</td>
<td>5:0</td>
</tr>
<tr>
<td>History of blood transfusion (+)</td>
<td>2 (18.2%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Positive for C100-3 antibody</td>
<td>6 (54.5%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Duration of illness (months)</td>
<td>45.0±80.3</td>
<td>116±115.7</td>
</tr>
<tr>
<td>sGPT before administration (K.U. (Mean±S.D.)</td>
<td>271.3±314.0</td>
<td>148.8±31.8</td>
</tr>
<tr>
<td>Serum 2-5AS activity before administration (pmol/dl (Mean±S.D.)</td>
<td>93.5±79.5</td>
<td>54.3±46.1</td>
</tr>
<tr>
<td>Total HA1 score (before administration, Mean±S.D.)</td>
<td>9.3±5.0</td>
<td>5.7±2.1</td>
</tr>
</tbody>
</table>

Absence of HCV antibody was not associated with the effects of IFN.

8. Anti-IFN antibody

Anti-IFN antibody was measured in all patients administered IFN at the end of the therapy, but it was not detected in any of the patients.

9. Side effects

Side effects or abnormal laboratory findings were noted in 7 of the 16 patients. They were fever in 4 patients, malaise in 2, headache and backache in 1 each leukocytopenia in 2, thrombocytopenia in 1, and elevated in serum GOT and GPT in 1.

The therapy could be continued except for the patient who showed elevates in serum GOT and GPT. Thrombocytopenia was resolved after the end of the therapy, but all the other side effects or abnormal laboratory findings soon disappeared during the therapy.

Discussion

We determined the method to administer IFN in consideration of changes in the 2-5 AS activity, which is an index of the anti-viral effect of IFN. According to Kawaguchi et al\(^\text{6}^\), the 2-5 AS activity in peripheral blood monocytes reaches a peak 1 week after the beginning of daily IFN administration but decreases despite continued IFN administration. Also the 2-5 AS activity is reported to be high level for 48 hours after the administration of IFN. From these findings, IFN was administered daily for the first week, then every other day for 6 months. IFN receptors of peripheral blood monocytes are reported to decrease gradually by daily IFN administration but not to decrease by intermittent administration\(^\text{7}^\). This also supports the advantage of IFN administration every other day.

Concerning the improvements of the liver function, the serum GPT level normalized in 11 of the 16 patients administered IFN, and it remained normal in these 11 patients even after the end of the therapy while it did not normalize in any of the patients in the non-IFN group. Therefore, the IFN therapy is considered to have been effective in our patients. Changes in the serum GPT level in IFN group could be classified into 5 types. Eight patients showed a type I pattern, in which the serum GPT normalized rapidly after the beginning of IFN administration and remained normal. The therapeutic effect of IFN against CHNANB is considered to be derived primarily from the direct antiviral action by the 2-5 oligoadenylate activity\(^\text{8}^\). If it is so, the liver function is expected to improve soon after the beginning of IFN therapy. In fact, the serum transaminase levels improved rapidly after the beginning of the IFN administration in 8 of the 11 patients in the normalized group. As shown in Fig. 8, the 2-5 AS activity in the normalized group was significantly higher than in non-normalized group at 1 week after the beginning of IFN administration. The initial high response of 2-5 AS activity to the IFN will be one of the predictive marker of favorable therapeutic results, but the results of therapy will be influenced by other factors such as the amount of
virus, various mediators induced by IFN.

In the patient shown in Fig. 4 who belonged to the normal group, the serum GPT level showed no improvements even after the beginning of IFN therapy. In this patient, the administration was discontinued 13 days after the beginning of the treatment, because of suspectable aggravation of tissue damage. However, the serum GPT level gradually decreased, and normalized 16 weeks after the end of the administration, and remained normal until 54 weeks after the beginning of the administration. Histological studies by liver biopsy during this period showed marked improvements in the histological profile to CIH. Nakano et al. observed that the expression of BMG on the hepatocyte membrane did not increase even after the IFN therapy in many patients with CHNANB and that the serum GPT level rapidly decreased after the beginning of IFN administration in such patients. They speculated that the serum GPT normalized rapidly, because IFN reduced the amount of the target antigen of virus-infected cells and prevented destruction of virus-infected hepatocytes by MHC-restricted T cell cytotoxicity. However, the expression of BMG on hepatocyte membrane was increased in a few patients after IFN administration, and the serum GPT level elevated slightly after the beginning of IFN administration in these patients. In the patient shown in Fig. 4, the serum transaminase levels improved after the end of IFN administration. Therefore, MHC-restricted T cell cytotoxicity may have played a major role in elimination of virus-infected hepatocytes in this patient. In this study the high serum BMG levels continued for a longer period in the non-normalized group than in the normalized group. This may be explained by an enhancement of the T cell cytotoxicity because of the high virus level in hepatocytes.

The therapeutic effects of IFN administration have been evaluated according to normalization of the transaminase levels and improvements in the histological profile of the liver, but are assessed recently also from virological approaches. Changes in the HCV antibody and HCV-RNA levels with the treatment have been introduced as new virological parameters.

In our present study, serum HCV-RNA was measured by the nested PCR method. HCV-RNA was found to be negative in 3 of the 6 patients in whom normalization of the serum GPT level continued even after the therapy. Serum HCV-RNA became negative quickly, often during or soon after the treatment, in patients whose serum GPT level normalized shortly after the beginning of IFN therapy (type I). These findings are considered to be important in determination of the dose and the period of IFN administration. However, HCV-RNA became temporarily negative but appeared again after completion of the treatment in some patients, or it remained positive while the serum GPT level continued to be normal after the therapy in others. New criteria for evaluation of the therapeutic effects using various markers including HCV-RNA are needed in such patients.

Since the serum GPT level elevated again in patients who remained or became positive for HCV-RNA, modifications are needed in the treatment of these patients.

Evaluation of the relationship between the therapeutic effects and the background factors of the patients revealed no clear associations. However, the age, the duration of illness, and the history of transfusion were suspected to be related to the effectiveness of IFN therapy. This point needs further clarification in a larger number of patients. Although the normalized group in this study included fewer patients with a history of transfusion, no difference was observed in the therapeutic effect according to the positivity or negativity for HCV antibody. More detailed investigations are needed concerning the relationship of the therapeutic effects with the pattern of infection or the HCV antibody level, i.e., the amount of infecting virus, as well as the presence or absence of HCV antibody.

In this study we classified the changes of transaminase level into five types after IFN administration and this type of classification has not been reported. The changes of transaminase will be reflected the event occurred in vivo, such as immune responses of the host or viral replications. So we consider that this
Effectiveness of Intermittent IFN Therapy

Our IFN therapy, in which IFN was administered daily for the first week and then 3 times a week, was shown to induce normalization of the serum GPT level in a very high percentage in patients with CHNANB. Moreover, HCV-RNA disappeared from the blood of some of these patients, suggesting the complete cure of type C hepatitis. This may eventually lead to prevention of liver cancer and is considered to contribute greatly to improving the prognosis of type C hepatitis. Furthermore, as this therapy causes no severe side effects, requires hospitalization for only 1 week at the beginning, and can be continued at the outpatient clinic, the reduced burden to the patients is considered to be another advantage of this therapy.

References


非A非B型慢性肝炎に対するヒト天然型インターフェロン-α
（HLBI）の6ヵ月間歇投与療法の有効性

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日野 照子 井出 達也 野口 誠司 渡邉 次 千
矢野 洋一 小野 勝之 村岡 晴雄 日野 和彦
谷川 久一

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（平成4年1月14日受理）

要 旨
非A非B型慢性肝炎（以下CHNANB）患者に対して、インターフェロンの長期、間歇投与を行い、肝障害の改善に対する有効性を検討した。
CHNANBと診断され、加療を必要とする患者26名を対象とし、16名にはIFN療法を行い、他の10名にはIFNを投与せず、1年間経過観察を行った。
IFNの投与量は1日1回3MU（一部1MU、6MU投与症例も存在）で、治療導入時の1週間は連日投与、その後引き続き週3回で6ヵ月外来にて筋注、投与終了後少なくとも6ヵ月以上経過観察を行った。
IFN投与群では投与開始後1年の時点で16例中11例（66.8%）で血清transaminase値の正常化をみた。この正常化群の中で、血清中のHCV-RNAを測定できた6例中3例（50.0%）がHCV-RNA陰性、もしくは陰性化が確認された。
正常化群では肝組織内の炎症所見が著明に改善していた、一方、IFN非投与群10例では全例肝機能の正常化が得られなかった。以上より本治療法は、CHNANBに対し優れた治療効果を示すと考えられた。

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