Pilot study of ofloxacin and interferon-alpha combination therapy for chronic hepatitis C without sustained response to initial interferon administration

Masafumi Komatsu MD, Tohru Ishii MD, Tsuyoshi Ono MD, Takao Hoshino MD, Tomoyuki Kuramitsu MD, Takashi Goto MD, Tomoo Fujii MD, Itaru Toyoshima MD, Mitsuro Chiba MD, Osamu Masamune MD

Since its introduction for non-A, non-B hepatitis and for hepatitis C (1-3), over 200,000 patients have been treated with interferon (IFN) therapy in Japan. Although this therapy has proven effective, the percentage of patients with long term normalization of transaminase levels is only 30% (4,5). Despite an initial response to IFN, some patients relapse after completion of IFN therapy. Some studies have suggested that such a relapse might be prevented by admini-
tration of larger dosages of IFN for longer periods (6); however, IFN treatment is costly and is associated with various adverse reactions. These factors have led to attempts at combination therapy with other agents. To date, combination therapy with ribavirin (7,8), ursodeoxycholic acid (9) and azidothymidine (10) have been reported. In 1993, Takada et al (11) reported that ofloxacin (OFLX), a new quinolone derivative, is effective against hepatitis C, and combination therapy with IFN enhanced therapeutic efficacy. We conducted a controlled trial using combination therapy with OFLX and IFN versus IFN monotherapy in patients with chronic hepatitis C (CHC) who underwent unsuccessful therapy with IFN.

**PATIENTS AND METHODS**

Twenty patients with CHC who had failed therapy with IFN were enrolled in the trial. All patients were positive for hepatitis C virus (HCV) RNA and had persistently abnormal liver function tests. Tests for the presence of antinuclear antibody and hepatitis B surface antigen were negative in all patients. Biopsy of the liver was performed within the six-month period before the start of IFN readministration. Histological studies found chronic hepatitis in all cases. Evaluation was made according to the Histology Activity Index of Knodell et al (12). Initial IFN administration was made more than six months before retreatment in all cases. Before retreatment, neither IFN nor other antiviral agents were administered.

Patients were assigned randomly to two groups – group A, which included 10 patients who received combination therapy with IFN and OFLX; and group B, which included 10 patients who received IFN only (Table 1). Recombinant IFNα-2b (Schering Plough, New Jersey) was administered at a dose of 10^6 U, six days per week for four weeks from initiation of therapy, and then at the same dose three days per week for 20 weeks. For group A, OFLX was administered for 12 weeks at a daily dose of 600 mg from the fifth week of IFN therapy.

Alanine aminotransferase (ALT) values were measured weekly for one month starting immediately before the start of IFN administration and monthly thereafter.

The HCV genotype was identified by polymerase chain reaction using the type-specific core region primer according to the method of Okamoto et al (13). Types 1, 2, 3 and 4 of the Okamoto et al classification correspond to types 1b, 2a and 2b of the Simmonds et al classification (14). Levels of HCV-RNA were measured by the multicyclic RT-PCR method (15) using the 5' non-coding region as a primer. The HCV genotype was determined before IFN treatment. HCV-RNA levels were measured six times – before IFN therapy; at one and four months of IFN therapy; at the end of therapy; and three and six months after therapy.

Antibodies to IFNα-2b were determined by bioassay just before IFN administration and one month after administration.

For statistical analysis, the Friedman repeated measures ANOVA on ranks test, the Mann-Whitney U test and Fisher's exact test were used. The results were expressed as the mean ± SD, and P<0.05 was considered statistically significant.

The study was approved by the institutional review board at Akita University School of Medicine, and written informed consent was obtained from all patients.

### Table 1

**Patient characteristics at the beginning of the trial**

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Group A (ofloxacin plus interferon)</th>
<th>Group B (interferon alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.8±9.6*</td>
<td>44.2±11.8*</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>8:2</td>
<td>7:3</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/mL)</td>
<td>95.5±48.0*</td>
<td>90.4±54.5*</td>
</tr>
<tr>
<td>Hepatitis C virus RNA titres</td>
<td>7.8±1.8*</td>
<td>8.2±1.2*</td>
</tr>
<tr>
<td>Hepatitis C virus genotype (1b:2a)</td>
<td>8:2</td>
<td>9:1</td>
</tr>
<tr>
<td>Liver histology, Histology Activity Index score*</td>
<td>7.9±3.6*</td>
<td>7.8±2.9*</td>
</tr>
<tr>
<td>Total dose of interferon in initial administration (IUx10^6)</td>
<td>554±65*</td>
<td>463±121*</td>
</tr>
<tr>
<td>Cases with undetectable hepatitis C virus RNA at the end of initial interferon administration</td>
<td>7 (70%)</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Cases with normalization of alanine aminotransferase levels at the end of initial interferon administration</td>
<td>7 (70%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Interval to retreatment from initial administration (days)</td>
<td>484±240*</td>
<td>558±270*</td>
</tr>
</tbody>
</table>

*Mean ± SD. Differences between groups A and B were not significant.*
RESULTS

In group A, serum ALT levels fell after one month of IFN treatment. After two months (after one month of OFLX combination therapy), until the end of the therapy, serum ALT levels were significantly lower than those before administration (P<0.01). Levels rose once after therapy but declined after six months. In group B, serum ALT levels began to decline after one month of therapy, and by six months were reduced significantly compared with pretreatment levels (P<0.05). Similar to results in group A, serum ALT levels rebounded transiently six months after completion of therapy. The decline in ALT levels was more marked in group A than in group B. Although no difference in serum ALT levels was present after the first month of IFN therapy, ALT levels in group A were significantly lower at two, four and six months (P<0.05) (Figure 1). No significant difference in the fraction of subjects whose serum ALT levels normalized after the first month of IFN therapy was observed. By the fourth month, ALT normalization rates for groups A and B were 90% and 40%, respectively. After the sixth month of therapy, the normalization rate in group A was higher than that in group B (Table 2). Moreover, the fraction of cases that maintained normal ALT levels six months after therapy was still higher in group A (40%) than in group B (10%).

HCV-RNA levels were significantly lower after the first month of IFN therapy for both groups (P<0.05). Levels continued to decline after four and six months of therapy. However, levels relapsed after completion of therapy in both groups and were lower in group A (P<0.05) (Figure 2). No significant difference in the rate of HCV-RNA clearance was observed. In fact, only two cases in group A remained HCV-RNA negative three and six months after therapy (Table 3).

Neutralizing antibodies to IFNα-2b were absent in all cases just before IFN therapy; however, neutralizing antibod-

![Figure 1](image1.png)

**Figure 1** Changes in serum levels of alanine aminotransferase (ALT) in patients treated with interferon (IFN) plus ofloxacin (OFLX) or IFN alone. Recombinant IFNα2b was administered for six months at a daily dose of 10^6 U. OFLX was administered for three months at a daily dose of 600 mg. Data presented as the mean ± SD. *P<0.05

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After 1 month</th>
<th>After 4 months</th>
<th>At the end of treatment</th>
<th>3 months after treatment</th>
<th>6 months after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin plus interferon (10 patients)</td>
<td>0</td>
<td>4 (40%)</td>
<td>9 (90%)</td>
<td>10 (100%)</td>
<td>4 (40%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Interferon alone (10 patients)</td>
<td>0</td>
<td>3 (30%)</td>
<td>4 (40%)</td>
<td>5 (50%)</td>
<td>2 (20%)</td>
<td>1 (10%)</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After 1 month</th>
<th>After 4 months</th>
<th>At the end of treatment</th>
<th>3 months after treatment</th>
<th>6 months after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin plus interferon (10 patients)</td>
<td>0</td>
<td>5 (50%)</td>
<td>8 (80%)</td>
<td>7 (70%)</td>
<td>2 (20%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Interferon alone (10 patients)</td>
<td>0</td>
<td>5 (50%)</td>
<td>7 (70%)</td>
<td>7 (70%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
therapy with IFN for HCV infection. No randomized, controlled trials have been performed to investigate this specific virus. Moreover, a therapeutic effect on HIV infection has been reported (19). Takada et al (11) provided the first report of increased rates of remission with combination therapy. IFN is administered for six months at a daily dose of 10^6 U. OFLX was administered for three months at a daily dose of 600 mg. Data presented as mean ± SD.

In the present study, serum HCV-RNA levels were not detectable at the end of initial IFN treatment in 50% of the patients. It is possible that this effect represents an initial response to IFN therapy. The total IFN dose in the initial therapy was over 400x10^6 U in all cases. Therefore, in the second trial we set the total dose at 840x10^6 U. In addition, to investigate the antiviral and synergistic effects of combination therapy with OFLX on transaminase levels, concomitant administration of OFLX was initiated one month after the start of retreatment and continued for three months. As a result, HCV-RNA levels at three months after the concomitant use of OFLX were lower than levels before the institution of OFLX. However, similar results were obtained with use of IFN alone. Thus, we were not able to verify the synergistic effect of combination therapy against HCV-RNA. On the other hand, we observed that two patients became HCV-RNA positive after discontinuing OFLX, despite continuation of IFN therapy. The rate of ALT normalization was higher in the combination group. Forty per cent of the patients maintained normal levels for more than six months after the end of IFN therapy. Among these, two cases demonstrated long term clearance of HCV-RNA. To verify these findings, larger clinical trials should be undertaken.

The mechanism of the effect of OFLX on HCV infection is not clear. Takada et al (11) reported that levels of HCV-RNA and ALT declined in three of nine patients with CHC to whom only OFLX had been administered (200 to 900 mg/day). Although favourable effects were reported in patients who were administered doses of 600 mg/day or greater, we set the dose of OFLX at 600 mg/day. This dose was limited by the development of diarrhea and anorexia in patients given 900 mg/day. As a result, mild diarrhea occurred in only one patient. In the combination therapy group, one patient demonstrated depression, which was alleviated by the suspension of IFN therapy and concomitant administration of antidepressant medication. Frequent reports of depression associated with administration of IFN are present in the literature. However, the contribution of OFLX is not known.

Neutralizing antibody to IFNα-2b was observed in one patient in the combination therapy group one month after the end of therapy. In that patient, a favourable decline in ALT levels was observed at the start of therapy. These levels rebounded after three months and normalized once again at the end of therapy, only to remain elevated thereafter. HCV-RNA was persistently positive in this patient. Roffie et al (23) demonstrated the emergence of a neutralizing antibody against IFN as a cause of the re-elevation of ALT levels – a so-called breakthrough occurring during IFN therapy. Because retreatment may simulate an effect, attention should be paid to the emergence of this antibody.

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REFERENCES


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