Clinical Study

Restoration of Innate and Adaptive Immune Responses by HCV Viral Inhibition with an Induction Approach Using Natural Interferon-Beta in Chronic Hepatitis C

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1. Introduction

About 180 million people (around 3% of the world’s population) are infected with the hepatitis C virus (HCV) [1]. Chronic hepatitis C (CHC) is a leading cause of chronic hepatitis, cirrhosis, liver failure, and hepatocellular carcinoma worldwide [2]. CHC is a serious global medical problem necessitating effective treatment. However, 50% of treated patients are not cleared of viremia when treated with pegylated- (PEG-) interferon- (IFN-) alpha plus ribavirin (RBV) for 48∼72 weeks (standard of care: SOC) [3, 4]. The triple combination of PEG-IFN-alpha, RBV, and a protease inhibitor (telaprevir or boceprevir) fails to eradicate HCV in approximately 20∼30% of treatment-naive and 50∼60% of treatment-experienced patients [5, 6]. Thus, more effective, more tolerable and/or more tailored therapies are required.

Viral kinetics in response to anti-HCV treatment is an important factor during treatment. With successful antiviral treatment, the HCV RNA concentration in serum promptly decreases to undetectable levels and remains negative throughout therapy and thereafter. The faster the virus becomes undetectable during therapy, the better the chance of achieving a sustained virologic response (SVR). Accumulating evidence suggests that an early response to treatment is best determined by the level of HCV RNA in serum at 4 and 12 weeks of therapy [7, 8]. Because an SVR has been shown to be more likely after favorable early viral kinetics (i.e., a more rapid and profound reduction in
HCV RNA levels), a rapid initial clearance augmented by induction therapy for the first several months was postulated as an approach to optimizing new therapeutic strategies to achieve SVR [9, 10]. HCV exists as a genetically heterogeneous viral population, named quasispecies. Thus, the clinical success of new HCV therapies will depend on their ability to suppress all viral variants as well as prevent the emergence of resistant viruses [11].

Recent advances in the understanding of innate immunity show that the activation of the innate immune system is essential for subsequent adaptive immune responses including specific antibody production and CTL activation which play a key role in protection against viral infections [12]. In addition to evading the innate immune system, HCV has evolved effective means of thwarting the adaptive immune system [13, 14].

IFNs are key mediators of the host innate antiviral immune response. IFN-stimulated gene (ISG) products can prevent the translation of viral RNA and thereby limit the initial viral spread in the liver until viral clearance occurs by HCV-specific T cells [15]. The first response is thought to be IFN-beta production by infected hepatocytes. IFN-beta has different signaling and biological activities from IFN-alpha and achieved a higher rate of viral clearance than IFN-alpha [16–21]. Contrary to the actions of IFN-alpha, IFN-beta and IFN-lambda signaling in the liver does not become refractory during repeated stimulation of the IFN signaling transduction pathway. The sustained efficacy of IFN-beta and IFN-lambda could be important for the treatment of patients who do not respond to PEG-IFN-alpha through a preactivated endogenous IFN system [21].

Resolution of an HCV infection may restore impairments of innate and adaptive immunity [22–24]. However, the issue of how to increase the initial virologic response rate has not been resolved and is complicated by viral breakthrough and adverse effects.

In a previous study, we have shown that cyclic and periodic IFN treatment (CPT) consisting of induction treatment (IT) with natural (n) IFN-beta for 2 weeks followed by maintenance treatment (MT) with nIFN-alpha for 2 weeks could prevent virologic breakthrough and achieve an early virologic response (EVR) and an end-treatment virologic response (ETVR). In addition to the improvement of innate immunity due to virologic clearance by CPT during the initial course of therapy, persistent virologic clearance and restoration of innate and adaptive immune responses by RBV plus PEG-IFN-alpha were more likely to result in a higher rapid virologic response (RVR), EVR, ETVR, and SVR. On the basis of these findings, we conducted a pilot study in 7 CHC patients with genotype 1b, high viral loads, and wild or intermediate type IFN sensitivity determining region (ISDR) to assess the efficacy, tolerability, and safety of treatment with RBV plus PEG-IFN-alpha 2b for 48 weeks (SOC) using an induction approach with initial virologic clearance induced by CPT for 24 weeks (novel combination treatment: NCT) [25].

Little is known about the chemokine and cytokine response to HCV infection before, during, and after IFN treatment. Aiming to better understand the immunological determinants of the protective immune response to HCV infection, we performed an extensive analysis of the innate and adaptive immune responses in CHC patients with genotype 1b and high viral load. We have evaluated the serum levels of cytokines and chemokines that mediate humoral and cellular immunity and inflammation, correlated with disease activity, and characterize the immunomodulatory effects of therapy.

In addition, we compared the efficacy and safety of NCT versus SOC in CHC patients with genotype 1b and high viral loads. The rate of SVR was significantly higher among patients receiving NCT than those receiving SOC. NCT is beneficial to treat difficult-to-treat CHC patients with genotype 1b and high viral loads.

2. Patients and Methods

2.1. Patients

2.1.1. Patients. Seven patients [3 males and 4 females, mean age 53.3 ± 8.5 years (range 39–66)] with CHC, genotype 1b (serotype 1), ISDR with 3 wild type, 3 intermediate type, and 1 not determined, and a viral load of 2144.3 ± 1701.2 KIU/mL (range 536–5000 KIU/mL) were enrolled in this open-label, prospective study. Patients underwent a liver biopsy before the IFN therapy, and the severity (inflammation grade and fibrosis stage) of liver disease [26] was evaluated as chronic hepatitis (grades 1–3, stages 1-2) (Table 1). Serum was collected from five healthy donors, ranging in age from 28 to 58 years. Written informed consent was obtained from all patients according to the Declaration of Helsinki.

2.1.2. Exclusion Criteria. The following were considered as exclusion criteria: refusal by women of child-bearing age or by sexually active patients to use a safe contraceptive, pregnancy or breast-feeding, cirrhosis with signs of decompensated liver diseases, coronary heart diseases, the presence of overt psychiatric diseases, active alcohol or drug abuse, uncontrolled diabetes mellitus, uncontrolled hypertension, uncontrolled retinopathy, autoimmune disorders, or any other unstable medical condition not because of liver disease. All patients were negative for hepatitis B surface antigen, and frequent causes of chronic liver diseases were excluded.

2.1.3. Study Design. Cyclic and periodic IFN treatment (CPT): the patients were treated with 6 cycles (24 weeks) of cyclic and periodic IFN treatment (CPT). One cycle of CPT consisted of IT with nIFN-beta (Feron, Toray, Chiba, Japan) at 6 MU/day, subcutaneously, for 3–6 MU/day, intravenously by drip infusion in 100 mL of saline solution, daily for 2 weeks followed by MT with nIFN-alpha (Sumiferon, Sumitomo, Osaka, Japan) at 6 MU/day, subcutaneously, three times weekly for 2 weeks.

CPT was followed by treatment with RBV plus PEG-IFN-alpha 2b (SOC) (novel combination treatment: NCT): we investigated the efficacy, tolerability, and safety of CPT for 24 weeks as induction therapy followed by RBV (Rebetol: Schering Plough, Kenilworth, NJ, USA; 200–800 mg/day, per os, daily) plus PEG-IFN-alpha 2b (Pegintron, Schering
Plough, Kenilworth, NJ, USA; 60–120 micro-g/day, percutaneously inj., once weekly) (SOC) for 48 weeks (total 72 weeks) in a pilot clinical trial as a potential treatment for 7 difficult-to-treat CHC patients with genotype 1b, high viral load (a viral load of more than 100 KIU/mL), and wild or intermediate type ISDR [25].

2.1.4. Measurements. All patients were monitored with clinical, biochemical, and virologic assessments before and every 1 to 4 weeks during the entire 72-week treatment period and were followed for an additional period of more than 24 weeks. The level of HCVRNA in serum was determined using the quantitative COBAS AMPLICOR HCV MONITOR test, or improved, a return to initial dosing levels was permitted. Safety was assessed with laboratory tests and an evaluation of adverse events (AEs) every 1–4 weeks during and after the end of NCT. A reduction in the RBV dosage from 800 to 200–600 mg per day and reduction in the PEG-IFN-alpha 2b dosage from 60–120 microg to 50–100 microg without virologic breakthrough were allowed to manage AEs or laboratory abnormalities that had reached predetermined thresholds of severity. If the AEs were resolved or improved, a return to initial dosing levels was permitted.

2.4. Statics. Data were expressed as the mean ± standard deviation, and a paired-t test was used to evaluate the differences of the means between groups, with a P value of <0.05 considered significant.

3. Results

3.1. Study 1. HCV viral titers decreased in all patients after 4 weeks of CPIT highlighting the efficacy of this treatment modality. None of the patients showed virologic breakthrough. Serum HCVRNA [2144.3 ± 1701.2 (range 536–500) KIU/mL at baseline] decreased significantly to 1.5 ± 2.4 KIU/mL ($P = 0.0157$) at the end of CPIT. The rates of RVR and EVR [partial EVR (pEVR), complete EVR (cEVR), and RVR plus cEVR (extended RVR)] were 7/7(100%) and 7/7 [100%; 4/7 (57.1%), 3/7 (42.9%), and 3/7 (42.9%)] respectively. Viral titers dropped below detectable levels in 5 patients before the end of CPIT, and in 2 patients

<table>
<thead>
<tr>
<th>Patient NO.</th>
<th>Age/gender</th>
<th>Body weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Liver histology (Stage/Grade)</th>
<th>ALT (IU/mL)</th>
<th>Outcome of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>baseline</td>
<td>24 weeks (end of NCT)</td>
<td>72 weeks (end of NCT)</td>
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<tr>
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<td>46.1</td>
<td>20.4</td>
<td>1/2</td>
<td>197</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
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<td>67.0</td>
<td>21.1</td>
<td>1/1</td>
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<td>28</td>
</tr>
<tr>
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<td>66/M</td>
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<td>3/2</td>
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<td>29</td>
</tr>
<tr>
<td>4</td>
<td>49/M</td>
<td>60.0</td>
<td>19.8</td>
<td>1/2</td>
<td>50</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>61/F</td>
<td>48.5</td>
<td>20.9</td>
<td>1/2</td>
<td>38</td>
<td>9</td>
</tr>
</tbody>
</table>

Early virologic responders

Late virologic responders

Mean ± SD: 53.3 ± 8.5

Table 1: Characteristics of chronic hepatitis C with high viral load, serotype-1 (genotype 1b), and wild or intermediate type in ISDR before, during, and after RBV plus PEG-IFN-alpha 2b using an “induction” therapy with cyclic and periodic interferon treatment (CPIT); novel combination treatment (NCT).
<table>
<thead>
<tr>
<th>Patient no.</th>
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<th>BMI (kg/m²)</th>
<th>Liver histology (stage/grade)</th>
<th>HCV-RNA (TaqMan PCR Log IU/mL)</th>
<th>ALT (IU/mL)</th>
<th>Hb (g/dL)</th>
<th>Platelet (10⁴/mL)</th>
<th>Outcome</th>
</tr>
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<td>NCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>61/F</td>
<td>48.5</td>
<td>20.9</td>
<td>A1/F1</td>
<td>5.9</td>
<td>38</td>
<td>12.1</td>
<td>17.7</td>
<td>SVR</td>
</tr>
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<td>46.1</td>
<td>20.4</td>
<td>A1/F1</td>
<td>6.2</td>
<td>197</td>
<td>9.2</td>
<td>17.5</td>
<td>SVR</td>
</tr>
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<td>47/M</td>
<td>67.0</td>
<td>21.1</td>
<td>A1/F1</td>
<td>5.9</td>
<td>37</td>
<td>14.8</td>
<td>14.3</td>
<td>SVR</td>
</tr>
<tr>
<td>4</td>
<td>49/M</td>
<td>60.0</td>
<td>19.8</td>
<td>A2/F1</td>
<td>6.6</td>
<td>50</td>
<td>15.0</td>
<td>22.0</td>
<td>SVR</td>
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<td>24.5</td>
<td>A2/F3</td>
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<td>15.7</td>
<td>9.6</td>
<td>SVR</td>
</tr>
<tr>
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<td>27.1</td>
<td>A2/F3</td>
<td>7.7</td>
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<td>13.8</td>
<td>39.1</td>
<td>TVR</td>
</tr>
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<td>58/F</td>
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<td>−/−</td>
<td>6.4</td>
<td>32</td>
<td>12.6</td>
<td>17.7</td>
<td>TVR</td>
</tr>
<tr>
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<td>73/M</td>
<td>53.4</td>
<td>21.0</td>
<td>A1/F1</td>
<td>6.4</td>
<td>20</td>
<td>12.1</td>
<td>22.4</td>
<td>SVR</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>58.9 ± 10.8</td>
<td>60.1 ± 11.6</td>
<td>21.9 ± 2.5</td>
<td></td>
<td>6.36 ± 0.61</td>
<td>55.1 ± 58.4</td>
<td>13.2 ± 2.1</td>
<td>18.9 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>SOC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>62/M</td>
<td>61.0</td>
<td>19.7</td>
<td>A2/F3</td>
<td>6.1</td>
<td>65</td>
<td>14.2</td>
<td>9.6</td>
<td>NVR</td>
</tr>
<tr>
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<td>61.3</td>
<td>19.6</td>
<td>A2/F2</td>
<td>6.8</td>
<td>96</td>
<td>16.0</td>
<td>16.4</td>
<td>TVR</td>
</tr>
<tr>
<td>3</td>
<td>69/F</td>
<td>48.2</td>
<td>21.4</td>
<td>A2/F2</td>
<td>7.4</td>
<td>54</td>
<td>12.0</td>
<td>12.9</td>
<td>NVR</td>
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<td>54/M</td>
<td>77.9</td>
<td>24.0</td>
<td>−/−</td>
<td>6.8</td>
<td>79</td>
<td>15.8</td>
<td>7.9</td>
<td>NVR</td>
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<tr>
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<td>64/M</td>
<td>71.4</td>
<td>25.0</td>
<td>A3/F2</td>
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<td>273</td>
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<td>13.9</td>
<td>SVR</td>
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<td>A1/F1</td>
<td>5.7</td>
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<td>TVR</td>
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<td>24.4</td>
<td>−/−</td>
<td>6.5</td>
<td>63</td>
<td>14.0</td>
<td>22.4</td>
<td>SVR</td>
</tr>
<tr>
<td>8</td>
<td>51/M</td>
<td>49.5</td>
<td>16.5</td>
<td>A1/F0</td>
<td>6.1</td>
<td>56</td>
<td>13.2</td>
<td>18.2</td>
<td>SVR</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>60.1 ± 6.9</td>
<td>62.9 ± 11.2</td>
<td>21.8 ± 3.0</td>
<td></td>
<td>6.59 ± 0.60</td>
<td>89.6 ± 76.5</td>
<td>14.4 ± 2.0</td>
<td>17.8 ± 6.1</td>
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</tr>
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</table>

after the end of CPIT (after beginning of RBV plus PEG-IFN-alpha-2b). The rates of ETVR at the end of CPIT and NCT were 5/7 (71.4%) and 7/7 (100%), respectively. The rate of SVR was 5/7 (71.4%). Transient virologic response (TVR) was found in 2 patients who showed undetectable HCVRNA in serum after the end of CPIT (Table 3).

To refine our understanding of the heterogeneity of therapeutic responses, patients were classified into two statistically distinct groups based on the time of clearance of viremia. Of note, early virologic responders (EVR) with undetectable HCVRNA in serum before the end of CPIT, which included 5 patients (pt. NO 1–5), showed SVR. Late virologic responders (LVRs), with undetectable HCVRNA in serum after the end of CPIT, which included 2 patients (pt. NO 6–7), showed a TVR. The viral titer values in LA VR in serum after the end of CPIT (Table 3).

Serum ALT decreased at the end of NCT and after the end of NCT. The rate of sustained biochemical response (SBR) was 5/7 (71.4%) (Table 1).

3.1.1. Serum Cytokines and Chemokines at Baseline (Figure 1). CXCL-8, CXCL-10, CCL-4, and CCL-11 levels were significantly higher (P < 0.05); IFN-gamma, TNF-alpha, IL-1alpha, IL-2, IL-6, IL-15, GM-CSF, G-CSF, and CCL-2 levels were higher; IL-10, IL-12, and IL-13 levels were significantly lower (P < 0.05) in all CHC patients than in the controls. IL-6, IL-15, CXCL-8, CXCL-10, and CCL-11 levels were significantly higher (P < 0.05), and IFN-gamma, TNF-alpha, IL-1alpha, IL-2, GM-CSF, G-CSF, CCL-2, and CCL-4 levels were higher in EVR than in the controls. IL-10 and IL-13 levels were significantly lower (P < 0.05), and IL-12 levels were lower in EVR than in the controls. GM-CSF, CXCL-10, and CCL-4 levels were significantly higher (P < 0.05), and TNF-alpha, IFN-gamma, IL-1alpha, IL-1beta, IL-2, IL-15, IL-6, IL-9, IL-4, G-CSF, PDGF, CXCL-8, and CCL-11 levels were higher in LAVR than in the controls.

3.1.2. Serial Values of Serum Cytokines and Chemokines during the NCT (Figures 2, 3, 4, 5, 6, 7, 8, and 9)

At the End of CPIT. In all CHC patients, the levels of CCL-4 decreased significantly (P < 0.05), the levels of IFN-gamma, TNF-alpha, IL-1alpha, IL-2, IL-4, GM-CSF, and G-CSF decreased, and the levels of IL-9, IL-10, IL-13, CCL-2, PDGF, and VEGF increased from baseline. In EVR, the levels of CCL-4 decreased significantly (P < 0.05) from baseline but to a lesser extent than in LAVR. The levels of CXCL-8 decreased in EVR but increased significantly (P < 0.05) in LAVR. The levels of CXCL-10, CCL-3, and PDGF decreased in EVR but increased in LAVR at the end of CPIT.

At the End of NCT. In all CHC patients, the levels of CCL-4 decreased significantly (P < 0.05), and the levels of IFN-gamma, TNF-alpha, IL-1alpha, IL-2, IL-4 (P < 0.1), IL-6, IL-9, IL-15, CXCL-8, CXCL-10 (P < 0.1),
Table 3: Effect of RBV plus PEG-IFN-alpha 2b using an “induction” therapy with CPIT (NCT) on serum HCV RNA in chronic hepatitis C with high viral load, serotype 1 (genotype 1b), and wild or intermediate type in ISDR.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Serotype</th>
<th>ISDR number of mutations in NS5A gene</th>
<th>Baseline</th>
<th>4 weeks (RVR)</th>
<th>12 weeks (EVR)</th>
<th>24 wks (end of CPIT)</th>
<th>72 weeks (end of NCT)</th>
<th>96 weeks (24 wks after end of NCT)</th>
<th>Virologic breakthrough</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>n.d.</td>
<td>1480</td>
<td>&lt;5.0</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>SVR</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>Wild (0)</td>
<td>824</td>
<td>&lt;5.0</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>SVR</td>
</tr>
<tr>
<td>3</td>
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<td>(+)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>SVR</td>
</tr>
<tr>
<td>4</td>
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<td>&lt;5.0</td>
<td>(+)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>SVR</td>
</tr>
<tr>
<td>5</td>
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<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
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<td>SVR</td>
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<td>&lt;5.0</td>
<td>(+)</td>
<td>(+)(−)</td>
<td>(−)(+)$</td>
<td>3400</td>
<td>(−)</td>
<td>TVR</td>
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<td>(+)</td>
<td>(+)(−)</td>
<td>(+)</td>
<td>850</td>
<td>(−)</td>
<td>TVR</td>
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<tr>
<td>Mean ± SD</td>
<td>I</td>
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<td>2298±1523</td>
<td>RVR EVR ETVR (CPIT) ETVR (NCT) SVR Without BT</td>
<td>7/7 7/7 5/7 7/7 5/7 7/7</td>
<td></td>
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chemokines (b), (b′) and chemokines (b), (b′) in chronic hepatitis C patients with high viral load, genotype 1b (serotype I), and wild or intermediate type of ISDR (all patients). RBV: ribavirin, PEG-IFN: pegylated interferon, CPIT: cyclic and periodic interferon treatment, NCT: novel combination treatment.

In all CHC patients, the levels of CXCL-10 decreased significantly (P < 0.05) from baseline. In LAVR, the levels of IFN-gamma, IL-1alpha, and CCL-4 decreased and the CCL-10 level significantly decreased (P < 0.01). CXCL-10 decreased significantly (P < 0.01) from the baseline in NCT and SOC after the beginning of treatment. HCV RNA levels decreased more in NCT than SOC (Figure 10).

CCL-3, CCL-11, PDGF, GM-CSF, and G-CSF decreased from baseline.

In EAVR, the levels of IFN-gamma, IL-1alpha, CCL-4, and CXCL-8 decreased significantly (P < 0.05), and the levels of CXCL-10 decreased (P < 0.1) from baseline. In LAVR, the levels of IFN-gamma, IL-1alpha, and CCL-4 decreased and CXCL-8 increased. The levels of IL-9, G-CSF (P < 0.1), and CXCL-10 (P < 0.1) decreased in EAVR but not in LAVR. The levels of IL-6, IL-12, IL-15, and CCL-3 (P < 0.1) decreased in LAVR but not in EAVR. CPIT induced the upregulation of IL-15 expression, but RBV/PEG-IFN-alpha 2b did not. IL-10 and VEGF levels increased in LAVR but were unchanged in EAVR.

**Four Weeks after the End of NCT.** In all CHC patients, the levels of IL-12 and VEGF increased significantly (P < 0.05), and IL-10 (P < 0.1) and CCL-2 levels increased from baseline. The levels of IFN-gamma, TNF-alpha, IL-1alpha, IL-1beta, IL-2, IL-4, IL-6, CXCL-8, CXCL-10 (P < 0.1), CCL-4 (P < 0.1), CCL-11, GM-CSF, and G-CSF decreased from baseline.

The levels of IL-12 and VEGF increased (P < 0.05) in EAVR, and to a lesser extent, in LAVR. The levels of IL-15 and CCL-2 increased in EAVR but decreased in LAVR. The levels of IL-13 increased (P < 0.1) in LAVR and to a lesser extent in EAVR. The levels of CXCL-10 (P < 0.1) decreased in EAVR and to a lesser extent in LAVR. CXCL-8 and CCL-3 levels were unchanged in EAVR but decreased in LAVR.

**Correlation of Serum Cytokine and Chemokine Levels to Therapeutic Responses (Figures 2, 3, 4, 5, 6, 7, 8, and 9).** The IL-15 level increased at the end of CPIT in EAVR and LAVR and 4 weeks after the end of NCT in EAVR but not in LAVR. The level of CXCL-8 decreased significantly (P < 0.05) in EAVR but not in LAVR during NCT. After the end of NCT, in EAVR but not in LAVR, the IL-12 level increased significantly (P < 0.05), and the CXCL-8 level decreased significantly (P < 0.05). CXCL-8 increased in LAVR at the end of CPIT and NCT.

At the end of NCT and after the end of NCT, the CXCL-10 level significantly decreased (P < 0.05) in EAVR but not in LAVR. At the end of CPIT and the end of NCT, the CCL-4 level significantly decreased (P < 0.05) in EAVR but not in LAVR.

3.2. Study 2. HCV viral titers significantly decreased (P < 0.05) from the baseline in NCT and SOC after the beginning of treatment. HCV RNA levels decreased more in NCT than in SOC (Figure 10).

The rates of early virologic response differed in the initial 4 and 12 weeks, and ETVR and SVR in CHC patients with genotype 1b and high viral loads treated with the NCT and
Figure 3: Effect of RBV plus PEG-IFN-alpha 2b using an “induction” approach with CPIT (NCT) on serum cytokines (a), (a’), and chemokines (b), (b’), in chronic hepatitis C patients with high viral load, genotype 1b (serotype I), and wild or intermediate types of ISDR (early virological responders). RBV: ribavirin, PEG-IFN: pegylated interferon, CPIT: cyclic and periodic interferon treatment, NCT: novel combination treatment. Significant difference: ∗∗P < 0.05, ∗∗∗P < 0.1.

Figure 4: Effect of RBV plus PEG-IFN-alpha 2b using an “induction” approach with CPIT (NCT) on serum cytokines (a), (a’) and chemokines (b), (b’), in chronic hepatitis C patients with high viral load, genotype 1b (serotype I), and wild or intermediate types of ISDR (late virological responders). RBV: ribavirin, PEG-IFN: pegylated interferon, CPIT: cyclic and periodic interferon treatment, NCT: novel combination treatment. Significant difference: ∗P < 0.05, ∗∗P < 0.1.
Figure 5: Effect of RBV plus PEG-IFN-alpha 2b using an “induction” approach with CPIT (NCT) on serum CCL-4 in chronic hepatitis C patients with high viral load, genotype 1b (serotype I), and wild or intermediate type of ISDR. RBV: ribavirin, PEG-IFN: pegylated interferon, CPIT: cyclic and periodic interferon treatment, NCT: novel combination treatment. Significant difference: *P < 0.05, **P < 0.1.

Figure 6: Effect of RBV plus PEG-IFN-alpha 2b using an “induction” approach with CPIT (NCT) on serum CXCL-8 in chronic hepatitis C patients with high viral load, genotype 1b (serotype I), and wild or intermediate type of ISDR. RBV: ribavirin, PEG-IFN: pegylated interferon, CPIT: cyclic and periodic interferon treatment, NCT: novel combination treatment. Significant difference: *P < 0.05, **P < 0.1.

4. Discussion

This study investigated the hypothesis that an induction approach using CPIT with nIFN-beta would increase the initial virologic response rate and restore innate and adaptive immune responses in CHC patients with genotype 1b and a high viral load. The rate of RVR in week 4, pEVR in week 12, cEVR (extended RVR) in week 12, virological response in week 24, and ETVR and SVR among CHC patients with genotype 1b and high viral loads receiving the SOC and NCT were significantly higher among patients receiving the NCT compared with patients receiving the SOC at week 12 (12.3 ± 1.2 versus 10.8 ± 1.8, P = 0.0641). Levels of Hb (13.2 ± 2.1 versus 14.4 ± 2.0 g/dL at baseline) in peripheral blood during the NCT and SOC in CHC patients with genotype 1b and high viral load were significantly higher among patients receiving the NCT compared with patients receiving the SOC at week 12 (12.3 ± 1.2 versus 10.8 ± 1.8, P = 0.0641).
HCVRNA leading to an improvement in innate and adaptive immune responses in difficult-to-treat CHC patients with genotype-1b, high viral loads, and wild or intermediate types of ISDR.

The current results (Figures 1, 2, 3, and 4) show that (1) the significantly lower levels \( (P < 0.05) \) of IL-12 and the significantly higher levels \( (P < 0.05) \) of CXCL-8, IL-10, CXCL-10, CCL-4, CCL-11, and VEGF in CHC patients compared to the controls at baseline suggested the impairment of innate and adaptive immunity in CHC patients, (2) the level of IL-15 was increased at the end of CPIT in both EAVR and LAVR; levels of CXCL-8, CXCL-10, CCL-4, and CCL-11 were significantly decreased \( (P < 0.05) \) in EAVR but not in LAVR during NCT, and (3) the level of IL-12 increased significantly \( (P < 0.05) \), and the level of CXCL-8 decreased significantly \( (P < 0.05) \) after the end of NCT in EAVR but not in LAVR. Importantly, the current study suggested that initial early virologic clearance induced by CPIT before the use of a combination of RBV and PEG-IFN-alpha 2b induced the restoration of DC function and improvement of activation of NK cells indicated by the upregulation of IL-12 and IL-15 and the downregulation of CXCL-8, CXCL-10, CCL-4, and CCL-11. These observations suggested the timing and breadth of innate and adaptive immune responses to be important in determining the outcome of HCV infections. Protective immunity against HCV likely depends primarily on the activation of both innate and cellular immune response [29].

Recent research identified multiple strategies that HCV employs to attenuate the innate type IFN response [30]. Innate immunity is the first line of defense against an invading viral, bacterial, or fungal pathogen, and the hepatitis C virus (HCV), a single-strand RNA virus, is no exception. The recognition of a viral pathogen via the a coordinated

Figure 7: Effect of RBV plus PEG-IFN-alpha 2b using an “induction” approach with CPIT (NCT) on serum IL-10 in chronic hepatitis C patients with high viral load, genotype 1b (serotype I), and wild or intermediate type of ISDR. RBV: ribavirin, PEG-IFN: pegylated interferon, CPIT: cyclic and periodic interferon treatment, NCT: novel combination treatment. Significant difference: \( * P < 0.05 \), \( ** P < 0.1 \).

Figure 8: Effect of RBV plus PEG-IFN-alpha 2b using an “induction” approach with CPIT (NCT) on serum IL-15 in chronic hepatitis C patients with high viral load, genotype 1b (serotype I), and wild or intermediate type of ISDR. RBV: ribavirin, PEG-IFN: pegylated interferon, CPIT: cyclic and periodic interferon treatment, NCT: novel combination treatment. Significant difference: \( * P < 0.05 \), \( ** P < 0.1 \).
interaction of the cells of the innate immune system leads to the activation of adaptive immunity targeting viral-specific antigens. HCV interferes with the activation of adaptive immune responses by innate immune cells [31].

There appear to be innate immune responses that control the levels of viruses and lead to significant decreases in the HCVRNA titer with SVR. The timing of these responses is not the same for early and late virologic responders. There is a distinct shift at the point at which viral replication begins to decrease in individual HCVRNA titers. One of the key characteristics of an HCV infection is the delayed immune response despite the early increase in the HCV titer and the induction of ISGs. The delay in the induction of the innate immune response that caused this decrease results in continued viral replication, which may account for the higher peak HCVRNA titers seen in the non-SVR group. This delay may lead to immune escape or exhaustion of the induced response due to high numbers of infected cells [32].

Chemokines and cytokines are critical regulators of liver inflammation, and innate and adaptive immunity to HCV, the complex orchestration of which is suggested to determine the outcome of HCV infections [30, 33].

Both maturation and functional differentiation of cDCs are altered during an HCV infection with decreased IL-12 [34] and increased IL-10 production in vitro [35, 36]. The HCV core protein has been shown to bind to the globular domain of the complement receptor of macrophages and DCs and downregulate IL-12 production [37]. Considering that IL-12 is a key cytokine in the induction of CD4 T-cell activation, whereas IL-10 has complex inhibitory effects, HCV-induced modulation of these cytokines may have special importance in altered HCV-specific T-cell responses in chronic HCV infections [30]. IL-12 governs the Th1-type immune response, affecting the spontaneous and treatment-induced recovery from HCV infection [38].

Increased levels of IL-15 at the end of CPIT suggested that initial viral clearance, induced by CPIT before the beginning of RBV plus PEG-IFN-alpha 2b therapy, improved the innate immune response to HCV.

IL-15 plays an important role in the innate immune system and is a stimulatory cytokine for DCs impaired in CHC. IL15 is induced by IFN-alpha and/or IFN-beta and stimulates the proliferation and accumulation of NK cells. IL-15 is required for the maturation and survival of NK cells. NK cells have roles in both innate and adaptive immunity [39]. The activation of NK cells, as well as the timing, breadth, and robustness of the subsequent antigen-specific T cell immunity, is likely to be substantially shaped by early events in the innate response to the pathogen. IL-12 and IL-15 are biomarkers for the innate immune response.

**Figure 9:** Effect of RBV plus PEG-IFN-alpha 2b using an “induction” approach with CPIT (NCT) on serum IL-12 in chronic hepatitis C patients with high viral load, genotype 1b (serotype I), and wild or intermediate type of ISDR. RBV: ribavirin, PEG-IFN: pegylated interferon, CPIT: cyclic and periodic interferon treatment, NCT: novel combination treatment. Significant difference: \( * P < 0.05, ** P < 0.1 \).

**Figure 10:** Changes in serum HCVRNA level during the NCT and the SOC in chronic hepatitis C patients with genotype 1 and high viral loads. NCT: novel combination treatment; cyclic and periodic IFN treatment (CPIT) consisting of induction treatment with natural interferon-\( \beta \) followed by maintenance treatment with natural interferon-\( \alpha \) for 24 wks as induction approach followed by SOC for 48 wks. SOC: standard of care; ribavirin plus pegylated interferon \( \alpha \) 2b for 48 wks.
High serum levels of IL-10 are associated with an incomplete response to IFN therapy. Chronic HCV infection is characterized by poor cellular immune response, which might be in part due to the production of immune suppressive cytokines like IL-10 [40–43]. IL-10 inhibits IFN-alpha production, promotes apoptosis of pDC, and downregulates effector T-cell responses [30]. IL-10-inhibiting peptides may have important applications to enhance anti-HCV immune responses by restoring the immunostimulatory capabilities of DCs.

The levels of CXCL-10, CCL-3, and PDGF decreased in EAVR but increased in LAVR at the end of CPIT. The level of CXCL-8 was significantly higher in CHC than in the controls. Because CXCL-8, the production of which is stimulated by HCV NS5A, is able to directly inhibit the antiviral activity...
viral genotypes [46–48].

announce the first-phase reduction of HCV viral load for all

Low CXCL-10 levels both in the liver and in plasma before

of CXCL-10 mRNA predicts the HCV viral kinetic response.

10 in chronic HCV infections. The intrahepatic expression

hepatocytes are likely the primary source of plasma CXCL-

concentration of the protein, indicating that HCV-infected

ment expression of intrahepatic CXCL-10 mRNA and plasma

CXCR 3 receptor and attracts T lymphocytes, NK cells, and

10 is a chemotactic CXCL chemokine. CXCL-10 targets the

significantly decreased in EA VR but not in LA VR. CXCL-

restoration of antiviral activity of type 1 IFN inhibited by

therapy [44, 45]. The levels of CXCL-8 decreased in EA VR

α

interferon

α

natural interferon-

for 24 wks as induction approach followed by

SOC for 48 wks. SOC: standard of care; ribavirin plus pegylated

α

for 48 wks as induction approach followed by

NCT and the SOC in chronic hepatitis C patients with genotype 1

and high viral loads. NCT: novel combination treatment; cyclic and

periodic IFN treatment (CPIT) consisting of induction treatment

with natural interferon-β followed by maintenance treatment with

natural interferon-α for 24 wks as induction approach followed by

SOC for 48 wks. SOC: standard of care; ribavirin plus pegylated interferon α 2b for 48 wks.

of IFN-alpha, higher levels of CXCL-8 in nonresponders

may contribute in part to the poor response to IFN-alpha

therapy [44, 45]. The levels of CXCL-8 decreased in EAVR

but increased significantly in LAVR. This result suggested

the restoration of antiviral activity of type 1 IFN inhibited by

CXCL-8 in EAVR but not in LAVR.

Serum CXCL-10 levels at baseline were higher in CHC

patients than in controls. Levels of serum CXCL-10 were

significantly decreased in EAVR but not in LAVR. CXCL-

10 is a chemotactic CXCL chemokine. CXCL-10 targets the

CXCR 3 receptor and attracts T lymphocytes, NK cells, and

monocytes. There is a strong association between pretream-

ment expression of intrahepatic CXCL-10 mRNA and plasma

concentration of the protein, indicating that HCV-infected

hepatocytes are likely the primary source of plasma CXCL-

10 in chronic HCV infections. The intrahepatic expression

of CXCL-10 mRNA predicts the HCV viral kinetic response.

Low CXCL-10 levels both in the liver and in plasma before

the onset of treatment are associated with SVR and pro-

nounce the first-phase reduction of HCV viral load for all

viral genotypes [46–48].

Serum VEGF levels at baseline were significantly higher

in EAVR but not in LAVR of CHC patients. Serum VEGF lev-

els were associated with SVR [49].

Serum CCL-4 and CCL-11 levels at baseline were higher

in CHC patients than in controls. Serum CCL-4 and CCL-

11 levels significantly decreased in EAVR but not in LAVR in

CHC patients. CCL-4-mediated T-cell infiltration is essen-

tial for the delivery of IFN-gamma to mediate protective
downstream responses against HCV infections in the liver.

It has been shown from the intrahepatic gene expression

profiles of chimpanzees that CCL-4 was upregulated during

acute infection at the time of viral clearance, but not in those

who failed to eradicate the virus [31]. CCL-11 is a chemokine

that is thought to selectively attract eosinophils by activating

CCR3 receptors. Several studies have shown that CCL-11 is

involved in the pathogenesis of inflammatory processes
during liver diseases as well [50]. Harvey et al. recently ana-

lyzed the association between chemokines and virologic re-

sponses to IFN and RBV in HIV and HCV coinfected patients

[51]; in patients achieving an SVR, plasma CCL-11 levels

before therapy were statistically higher than in nonrespond-

ers [31].

Study 2 has shown that NCT was well-tolerated and

enhanced RVR, cEVR (extended RVR), ETVR, and SVR rates

in difficult-to-treat CHC with genotype 1b and high viral

load and revealed less adverse effects (AEs) than those in

SOC. The higher virologic response rates highlight the ben-

efit of NCT with an induction approach using nIFN-beta in

CHC patients.

These results suggested that (1) early virological clear-

ance by CPIT before the beginning of RBV/PEG-IFN-alpha

2b treatment induced the restoration of innate immune re-

sponses and lead to antiviral responses and (2) persistent viro-

logic clearance for more than 48 weeks with the subsequent

RBV plus PEG-IFN-alpha 2b therapy-induced restoration of

innate immune responses linked to adaptive immune

responses and resulting in SVR and SBR. These results sug-

gested that CPIT improved the innate immune response;

however, there was insufficient improvement of the adaptive

immune response in CHC during NCT. The findings from

this study support the concept that viral clearance early in

the course of therapy with reduced virologic resistance is

linked to restoration of innate and adaptive immune re-

sponses, suggesting that agents providing the greatest viral

suppression leading to extended RVR may be preferable as

the initial early induction approach. An initial viral clearance

induced by more adequate CPIT before beginning RBV plus

PEG-IFN-alpha 2b therapy may lead to an improvement of

innate and adaptive immune responses resulting in a higher

rate of SVR in difficult-to-treat CHC patients with genotype

1b and a high viral load.

In previous studies, dose reductions or treatment dis-

continuations of PEG-IFN-alpha that were often required to

manage adverse hematological events have been associated

with a reduction in therapeutic efficacy. In NCT, no serious

AEs were found, and good tolerance of NCT was confirmed

by the high compliance rates. Indeed, the results observed in

this study agree favorably with other findings on the safety of

IFN-beta treatment in CHC patients and support the use of

nIFN-beta as a safe and alternative option.

5. Conclusion

An induction approach with nIFN-beta for 24 weeks fol-

lowed by SOC for 48 weeks (NCT) was well tolerated with-

out discontinuation. NCT prevented viral breakthrough

with viral clearance leading to an enhanced early virologic

response and improved SVR rates in difficult-to-treat CHC

patients with genotype 1b and high viral loads. Early viro-

logic clearance (extended RVR) by CPIT for 24 weeks

before the beginning of RBV plus PEG-IFN-alpha treatment

induced the restoration of innate immune responses linked
to adaptive immune responses and resulting in SVR and SBR. The higher SVR rates in CHC patients with genotype 1b and high viral loads among patients receiving NCT compared with SVR rates in those receiving SOC were revealed. NCT is beneficial to treat difficult-to-treat CHC patients with genotype 1b and a high viral load.

Conflict of Interests

The authors declare that they have no conflict of interests.

References


